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THE DEVELOPMENT OF *FOMES CAJANDERI* KARST. IN NATURE AND IN CULTURE

by

© WILLIAM MANNING WONG

A THESIS

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ABSTRACT

The growth and development of *Fomes cajanderi* Karst. in nature and in culture was investigated. In the early stages of wood decay the hyphae are thin-walled, hyaline, clamped, and sparse, growing in a longitudinal direction in the tracheids. In the advanced stages of decay the hyphae are found in dense aggregations near the ray cells. The development of the natural basidiocarps has been followed from the primordium to the sporulating stage. The primordium consists of generative and skeletal hyphae growing outwards in a radial manner to form a hemispherical structure. In the basidiocarps the marginal region is composed of generative hyphae and developing skeletal hyphae. The context region consists of irregularly thickened generative hyphae and fully developed skeletal hyphae. The differentiated generative hyphae are found in "islands" among the parallel aligned skeletal hyphae. The dissepiments are formed by the downward growth of interwoven generative and skeletal hyphae.

Three types of hyphae were consistently found in agar, still (liquid), and wood-block culture. They are the thin-walled, nodose-septate hyphae, the irregularly thickened, nodose-septate hyphae, and the fiber hyphae. The effect of various carbon sources on the amount of mycelial growth was examined and it was found that the best growth occurred with cellobiose and D-mannose while little growth occurred

with D-fructose, D-xylose, galactose, and sucrose. Formation of fertile basidiocarps occurred in all three types of cultures. Observations of the cultural basidiocarps revealed the same three types of hyphae as those found in natural basidiocarps. Differences in the basidiocarp appearance and hyphal organization were noted, but the form and mode of development of the hyphae were the same.

At present there are two sets of terms used to describe hyphal morphology of the natural and cultural mycelium in the polypores. Corner's terms are used to describe the structure and development of the natural basidiocarps. Nobles' terms are used to describe the structure of the cultural mycelium. With *F. cajanderi* there is uniformity of development of the hyphae through the vegetative and reproductive phases in nature and in culture. I believe that one set of terms is sufficient for description of the mycelium and that the terms generative, irregularly thickened generative, and skeletal hyphae should be used to describe the development of the mycelium and basidiocarp in the natural and cultural environment. This simplification of terminology would make comparison of the structure and development of different species much less difficult and thus serve to clarify interrelationships between species of the Polyporaceae.

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INTRODUCTION

The Polyporaceae is a large, economically important family of fungi recognized as major wood-decaying organisms. The biology of many species has been extensively examined, especially the mycelial development in wood and the macroscopic features of the basidiocarps. The decay of wood products by these fungi has been the stimulus for numerous studies to find methods of preventing such damage. Investigations have disclosed many genetical, physiological, and biochemical aspects of the life history of polypores and descriptions of the basidiocarps are found throughout the literature, since these structures are the most distinctive part of the fungus.

For many years mycologists have been concerned with establishing stable taxonomic relationships between various members of the Polyporaceae. Macroscopic characters were used primarily in delimiting the taxa and, because of the polymorphic nature of the basidiocarps, a number of artificial classification systems were constructed. The taxonomic position of many polypore species has still not been resolved, and no classification system for the Polyporaceae can be regarded as generally accepted (Bondartseva, 1961). Corner (1932a, 1932b, 1953) was the first investigator to study the microstructure of natural basidiocarps in detail. His work emphasized the structure and development of hyphal types in polypore basidiocarps

and he introduced the "mitic" system. In this system there are three hyphal types which, in various combinations, make up the complex microstructure of the basidiocarps. The three hyphal types recognized were the generative hyphae, skeletal hyphae, and binding hyphae. Cunningham (1954, 1965) used this "mitic" system to establish a classification based on hyphal types present in the basidiocarps. Unfortunately, Cunningham set up his classification using only a limited number of species and sometimes made incorrect observations which resulted in an artificial system of classification.

The first major investigation of the development of wood-decaying fungi in culture was done by Long and Harsch (1918). These workers grew a large number of fungi on a variety of media and described the macroscopic and microscopic characters of the cultures. Basidiocarps frequently formed in culture and they noted that "the size, shape, and color of the pores and tubes produced in artificial cultures on many of the agars are practically identical with those found in nature for a given species."

The morphological studies of Nobles (1948, 1958, 1965) have been the major works used to identify cultures of wood-inhabiting polypores. Basing her key on both macroscopic and microscopic characters produced in culture, she has separated various groups by a number of criteria. These are the absence or presence of extracellular oxidase, septation of the hyphae, occurrence of special structures

and spores, color of the hyphae and mycelial mats, changes in agar color, rates of growth, formation of fruit bodies, odor, host relationships, and interfertility phenomena. She believes that these cultural characters can provide a guide to relationships in the Polyporaceae.

These various studies have investigated the vegetative mycelium, the reproductive structures produced in culture, in some instances, and the structure of the natural basidiocarps. Practically no work has been done involving comparison of the development of hyphal types in cultural and natural basidiocarps. For most species detailed hyphal analyses of the basidiocarp have not been carried out. Usually only brief accounts of hyphal morphology of the basidiocarps are provided in species descriptions and no information on hyphal development is given. The vegetative mycelium in culture has been extensively studied, but the development of basidiocarps in culture has received little attention. The investigations of Plunkett (1956, 1958, 1961) using *Polyporus brumalis*, a stipitate polypore, have provided much of what is known of the effect of the physical environment on basidiocarp morphogenesis. However, the microscopic characters of basidiocarps formed in culture were not examined. Nobles (1971) has pointed out that in cultures of all species of wood-inhabiting Hymenomycetes, including those with basidiocarps that are difficult to dissect and whose hyphal systems are difficult to determine, generative hyphae and hyphae

modified to form the characteristic microstructures of the species may be readily observed.

The lack of information concerning growth and development of these fungi prompted me to carry out a detailed study of the development of *Fomes cajanderi* Karst. in nature and in culture. This species was chosen for three reasons. Firstly, the species is fairly common and widespread in Alberta so that fresh specimens could be readily obtained. Secondly, the great propensity of *F. cajanderi* to fruit in culture provided a good opportunity to compare hyphal development in the natural and cultural basidiocarps. Thirdly, the closely related *Fomes roseus* (Alb. and Schw. ex Fr.) Karst. is often confused with *F. cajanderi* as they both have a pink-colored pore surface. The objectives of this study were: (1) to provide basic information needed to understand microstructural changes fundamental to morphogenesis in this species and (2) to evaluate the effects of selected factors of the cultural environment on growth.

There were three approaches to this study. The first was a detailed hyphal analysis of the natural basidiocarps to determine the structure and mode of development. Growth from the vegetative hyphae in the wood to formation of fully developed basidiocarps was examined. The second approach was an investigation of the vegetative and reproductive development of the fungus in three types of culture: agar culture, still (liquid) culture, and wood-

block culture. The third approach involved exploratory physiological work examining growth rates at various temperatures, carbon utilization, and decay of wood-shavings supplemented with L-asparagine. A search of the literature showed no carbon utilization studies or wood-decay experiments on this species.

The results of these studies will be presented in two major sections. The first will be a detailed account of the growth of the vegetative mycelium and basidiocarp development of *F. cajanderi* in nature. The second section will describe the growth and development of the fungus in the three types of cultures. The physiological results will also be presented in this section. The results and observations will be brought together in the discussion to present a complete view of the development of *F. cajanderi* in nature and in culture.

LITERATURE REVIEW

The basidiomycete *Fomes cajanderi* Karst. belongs to the large family Polyporaceae, an economically important group of mainly wood-decaying fungi. The family is characterized by the hymenial layer lining the inner surfaces of regular or irregular tubes or lamellae. The classification of the Polyporaceae is presently undergoing extensive revision as more studies are being conducted on the polypores. No classification proposed so far has been universally accepted. A brief review of the major classifications currently in use is given.

The classification of Overholts (1953) incorporates mainly macroscopic features for delimiting genera and species of North American polypores. Overholts uses basidiocarp shape, color, texture, pore layer appearance, and substrate preference as major criteria. Microscopic features such as spore size and shape and hyphal appearance are also used for identification. The classification lists 8 genera in the Polyporaceae with the genus *Fomes* containing about 40 species. His concept of the genus *Fomes* is based on the perennial nature of the sporophore and the tube region consisting of several distinct or indistinct layers to which additions are made yearly.

Lowe (1957) gave an account of the genus *Fomes* in North America in which 68 species are treated, grouped in 5 sections. The sections are based on context color and spore

appearance. Species are keyed out mainly by microscopic features such as absence or presence of setae and spore size and shape. *F. cajanderi* is placed in a section characterized by a pink or rose context and hyaline spores.

Bondartsev (1953) has written the largest single volume on the polypores to date. His book encompasses almost 300 species and about 200 of their varieties and forms. A natural classification is proposed for the Polyporaceae (*sensu stricto*) consisting of 5 subfamilies and 10 tribes. The groups are keyed out by a number of criteria including basidiocarp shape, tissue color and consistency, absence or presence of cystidia or setae, spore shape, size, and color and substrate preference. A detailed synopsis of this classification first appeared in "Annales Mycologici" (Bondartsev and Singer, 1941). In the classification, *F. cajanderi* (as *Fomitopsis subrosea* (Wier) Bond. and Sing.) is placed in the subfamily Fomitoideae and the tribe Fomiteae. Features of the tribe Fomiteae include oblong, colorless spores, diversely colored corky to woody tissue, stratified tubes, absence of setae, and usually an ungulate pileus covered by a crust.

Nobles (1958, 1965) was the first worker to use the cultural features of the mycelium in a classification scheme. In her key, the first division is based on the presence or absence of extracellular oxidase in culture. The presence of the enzyme was detected by growing cultures on gallic or tannic acid agar or by dropping an alcoholic

solution of guaiacum on the mycelial mat. The rapid appearance of blue color indicated the presence of the enzyme. After the first division was delimited, subsequent groups were keyed out by types of septation, hyphal types, fruiting in culture, color of mycelial mats, odor, growth rates, occurrence of oidia or chlamydospores, host relationships, and interfertility phenomena. Nobles noted that two major groups could be distinguished in the Polyporaceae. One group, considered primitive, consisted of species that produced no extracellular oxidase and, if heterothallic, showed the bipolar type of interfertility. The second group was considered advanced and consisted of species that produced extracellular oxidase and, if heterothallic, showed the tetrapolar type of interfertility. *F. cajanderi* was reported to be among the primitive group of polypores.

Fomes cajanderi Karst.

1. Classification and distribution

In the history of the classification of *Fomes cajanderi* Karst. there is much confusion and conflicting information. The problem arose due to the ambiguous use of the name *Trametes carnea* Nees by early American workers to describe a pink to rose-colored polypore found in North America. *T. carnea* was first described as a new species from Java in 1826 by Blume and Nees von Esenbeck as *Polyporus carneus* (Weir, 1923). Fries (1838) also used the name *P. carneus* and described the fungus. The Javanese fungus was then

confused by Fries (1874) in *Hymenomycetes Europaei* with a similar polypore recorded in America by Berkeley. Unfortunately, the original specimens of Blume and Nees von Esenbeck have not been compared with the American collections.

In 1847, Berkeley described a new species of polypore from Ceylon as *Polyporus rubidus*. Material of this species had also been found in Java, Australia, and the Philippines and its description agreed well with *P. carneus*. Both Weir and Bresadola (1912) believed that *P. rubidus* was a synonym of *P. carneus*. Weir reported that specimens of *P. rubidus* are entirely distinct from the polypore found in North America, differing in basidiocarp color, in spore shape and size, in the character of the pileus, in decay, and in substrate preference.

Berkeley had in his herbarium specimens of *F. cajanderi* which were collected in North America in 1857-58 and were labelled *Polyporus palliseri* and *Trametes arcticus*. These names were not published by Berkeley, although Cooke in 1881 published the name "*Polyporus palliseri* Berk. in Herb. Berk." (Weir, 1923). Since the above names were not accompanied by descriptions, they can be disregarded.

Due to the confusion associated with the name *Trametes carnea*, Weir (1923) proposed a new name, *Trametes subrosea*, for the American fungus. Overholts (1933) transferred the species to the genus *Fomes* and later gave a description of *Fomes subroseus* (Weir) Overholts (Overholts, 1935). However, Karsten (1904) had earlier named and described the

species as *Fomes cajanderi*, unknown to Weir and Overholts. Thus Karsten's name has priority, since Weir's name was published in 1923.

Bondartsev and Singer (1941) placed the species in the genus *Fomitopsis* because of the corky consistency of the tissue and the pinkish or pink to light brown color. In Bondartsev's major work "*The Polyporaceae of the European USSR and Caucasia*" (1953) a detailed description of *Fomitopsis subrosea* (Weir) Bond. and Sing. is given.

Since most workers today (Lowe, 1956, 1957; Nobles, 1965; Lowe and Gilbertson, 1961a, 1961b; Adams and Roth, 1967, 1969; Neuhauser and Gilbertson, 1971) recognize the name proposed by Karsten, *F. cajanderi* will be used in this thesis.

F. cajanderi is widely distributed throughout the north temperate zone. Lowe (1957) reports that in North America, the species has been collected from Quebec to Florida westward to California and British Columbia. Overholts (1953) lists an extensive number of states and provinces from which he has examined collections of the species. It is found in Northern Europe and collections have been made in Siberia and the Far East (Bondartsev, 1953).

Zeller (1926) reported the species (as *T. subrosea*) as being a predominate wound parasite of stone-fruit orchards in coastal California and British Columbia. The fungus produced a brown-pocket rot of the heartwood.

Lowe (1957), Lowe and Gilbertson (1961a, 1961b), and

Overholts (1953) record *F. cajanderi* as a brown-rot fungus occurring on the wood of gymnosperms or rarely on angiosperms. Korstian and Brush (1931) reported that the species was by far the most important one found destroying the heartwood of southern white cedar in the Eastern United States. Bondartsev (1953) listed larch and spruce and more rarely fir and pine as major substrates of the species in Europe and Siberia.

2. Basidiocarp structure

Although *F. cajanderi* is widely distributed, no studies have been done on the morphology of the species. The information available on structure is found in species descriptions based on basidiocarps considered to be mature. Lloyd (1915), who called the fungus *T. carnea*, noted that in the Eastern states the pileus surface was even and often pale, while in the West it was more fibrillose, uneven, and dark. Some of the specimens were thickened with an additional indistinct pore layer.

Weir (1923) described the pileus as "usually thin but sometimes conspicuously stratified when old, normally zonate with radiating appressed fibrils..., brown, brownish pink, silvery gray or black with age." He also noted that the darker colored context, the conspicuous narrow zonate and radiate fibrillose surface, and the narrow elongate, allantoid spores of *F. cajanderi* (as *T. subrosea*) distinguished it from the closely related, similarly colored *Fomes roseus* (Alb. and Schw. ex Fr.) Karst.

Overholts (1953) gave a detailed description of the macroscopic features of *F. cajanderi* (as *F. subroseus*). The major characters were the applanate or occasionally convex or ungulate pileus, the pinkish-red to pinkish-brown, dirty rufescent, grayish-brown, grayish, and, in old specimens, blackish pileus surface, the glabrous or radiately fibrillose or rugulose, often zonate surface, the rosy-pink to pinkish-brown floccose to soft-corky context, and the concolorous, indefinitely stratified tubes. The basidiospores were narrow-cylindric, slightly curved, hyaline, and measured 4-7 x 1.5-2 μ. The hyphae when stained in KOH were pale brown and long and flexuous, with no cross-walls or clamp connections.

Lowe (1934, 1957) gave a description similar to that of Overholts. In his account, Lowe briefly described the hyphae of the basidiocarps, stating "context hyphae rarely branched, thin- to moderately thick-walled, non-septate, mixed with a small amount of thin-walled clamped hyphae, 3-5 μ in diameter; tramal hyphae similar."

The Russian worker Bondartsev (1953), who used the name of *F. subrosea*, described the fruiting bodies as tough coriaceous, thin, flat, sessile or slightly effused-reflexed, often imbricate or laterally fusing as into oblong bands. The surface was zonate or almost azonate, occasionally striated, with radially flattened fibers, pinkish-fuscous, silver-gray to black on old parts. The tissue was corky and flocculent, and the tubes were

filled with a white substance. The hyphae were faintly colored, rusty to light-brown in bulk, with very sparse branchings, thick-walled or even devoid of lumen, in the tubes the hyphae were generally thinner and more sinuous. The spores were colorless, cylindrical, slightly flexed, and rostellate at the base, 5-6.5 (7) x 1.5-2 μ in size.

Bondartsev notes that all specimens of the fungus examined (except for a very large one) proved to be one-layered. He concludes that stratification occurs very rarely which probably accounted for the classification of the species in the genus *Trametes* by other authors.

3. Vegetative mycelium

Overholts (1953) remarks that *F. cajanderi* (as *F. subroseus*) has been so confused with *F. roseus* that it is difficult to draw reliable conclusions from the literature. Part of this confusion is due to the similar appearance of the decay. Both species produce a brown cubical rot. Information on the mycelium in the wood is sparse; in the species descriptions already referred to, the authors only mention that the fungus produces a brown rot.

Hubert (1924) investigated the decay of wood by a number of fungi and reported that *F. cajanderi* (as *T. carnea*) in the heartwood of white spruce produced a brown rot. Microscopic examination of the decayed wood showed that the hyphae penetrated all areas of the cell walls and the bore holes formed were much larger than the hyphae; no constriction of the hyphae occurred as they passed through the walls.

Zeller (1926) observed that the brown cubical rot produced by the species was more or less restricted to definite pockets in the wood, hence the name brown-pocket rot. The individual pockets contained a brown, punky wood which crumbled easily and was usually shrunken and cracked into cubical form.

Studies by Korstian and Brush (1931) revealed that the decay in southern white cedar was confined to the heartwood of both the butt and the trunk. In the butt, the rot may extend into the brace roots while in the trunk, decay was frequently confined to definite areas in the wood, forming pockets. Also in the earlier stages, the wood was a light reddish-brown, later becoming darker and breaking up into more or less rectangular blocks which crumbled under pressure. Microscopic features of the decay were not noted.

In the Pacific Northwest the principal heart-rot fungus attacking young, glaze-damaged Douglas fir is *F. cajanderi* (Adams and Roth, 1969). The transverse breaks in the main stem of the trees became almost exclusively infected by *F. cajanderi*, the decay of the living trees being confined to the heartwood by a hollow-cylinder of apparently decay-resistant heartwood.

The cultural aspects of the vegetative mycelium have not been closely examined. In the few papers mentioning growth in culture, only brief descriptions are given. For example, Snell (1923a) described one culture of *F. cajanderi*

(as *T. carnea*) as showing little initial soft, white mycelial growth and soon forming a tough matted rose-colored mycelium on agar and wood. He also reported that the delicate white mycelium spread rapidly over wood blocks but never fruited while the rose-colored mycelium did not readily infect the wood blocks but usually formed fruiting bodies.

The consistency and color of the mycelial mats was variable as noted by Snell, Hutchinson, and Newton (1928). One culture was described as "bunchy tomentose like washed cotton flannel and powdery when worked with a needle" while other cultures formed a tough mycelial skin. These investigators also found that the tougher the mycelial skin, the more pronounced the color became.

Nobles (1958, 1965) described the species growing on 1.25% malt extract agar as forming cottony to cottony-floccose and pitted mats, pink or vinaceous, with poroid fruiting areas forming over most of the surface after 6 weeks. Chlamydospores were rare, thin-walled, and ovoid to elongate. The hyphae were of the fiber type and nodose-septate type. The fiber hyphae were aseptate, occasionally branched, and thick-walled with lumina narrow or apparently lacking. The nodose-septate (clamped) hyphae were thin-walled or irregularly thickened with a narrow, sinuous lumen and enlarged clamp connections. Basidiospores that formed in culture were similar in size and shape to those of the natural basidiocarps.

4. Genetical studies

Mounce and Macrae (1937) were the first workers to report that *F. cajanderi* (as *F. subroseus*) was heterothallic and bipolar. Monosporous cultures were obtained from basidiospores released from sporophores grown on prune or malt agar or on small blocks of Douglas fir. The results of this early study were confirmed by Neuhauser and Gilbertson (1971). Mounce and Macrae also demonstrated the distinction between *F. subroseus* and *F. roseus* (a closely related polypore) by interfertility mating tests and differences in spore shape and size.

Adams and Roth (1967) studied paired cultures of *F. cajanderi* and showed that the dark lines of demarcation that formed at the interface of colonies provided a reliable basis for distinguishing genetically distinct dikaryon mycelia. Paired cultures that were distantly related formed demarcation lines 95-100% of the time while the closest related cultures formed lines about 50% of the time. Using the demarcation lines, Adams and Roth (1969) further demonstrated that in 49 glaze-damaged Douglas fir trees, there was an average number of 2.3 genotypes of *F. cajanderi* per tree.

5. Physiological studies

In the majority of papers reviewed, *F. cajanderi* was only superficially examined along with a number of other wood-decay fungi. The uncertainty regarding the identity of *F. cajanderi* and *F. roseus* reduces to some extent the

value of physiological data obtained by early workers. For example, Snell (1922) studied the physiological relations of the basidiospores, mycelium, oidia, and chlamydospores of five basidiomycetes, including *F. roseus*. The effect of temperature, light, media, and drying on spore germination was investigated as well as the growth of mycelium at different temperatures. One year later, Snell (1923a) stated that the *F. roseus* cultures described and used in his previous paper were actually cultures of *F. cajanderi* (as *T. carnea*).

Field observations by Snell, Hutchinson, and Newton (1928) on the moisture requirements for fruiting revealed that basidiocarps of *F. cajanderi* (as *T. subrosea*) were collected in relatively moist situations such as on logs covered with bark or on wood in ravines, near brooks or waterfalls. The same preference for relatively moist conditions was also noted in wood block cultures in flasks. *F. cajanderi* generally fruited in the lower portions of the flasks where it was moister as compared to *F. roseus* which always fruited higher in the flasks.

Snell, Hutchinson, and Newton (1928) found that different growth rates at 30°-32°C allowed absolute differentiation between *F. cajanderi* (as *T. subrosea*) and the closely related *F. roseus*. Maximal radial growth of *F. cajanderi* occurred at 30°C while *F. roseus* grew slower and showed optimal growth at 26-28°C. The maximum temperature at which growth of *F. cajanderi* was inhibited was 38°C. These

two cardinal temperatures were similar to those found by Humphrey and Siggers (1933).

Snell (1923b) subjected wood block cultures of *F. cajanderi* (as *T. carnea*) and four other wood-decay fungi to various temperatures to determine their resistance to moist and dry heat. He found that *F. cajanderi* showed little growth after 1/2 to 1 day of dry heat at 90°C. In moist heat, some growth occurred after 24 hours of treatment at 46° and 48°C, but only slight growth developed after 52°C for 12 hours.

Zeller (1926) demonstrated that the brown rot caused by the fungus in peach and prune trees was produced by the enzymes ligninase, cellulase, and hemicellulase. He also found emulsin present in the mycelium.

Levi and Cowling (1969) examined the relative cellulolytic activities of a number of brown-rot fungi, including *F. cajanderi* (as *F. subroseus*). The results were very surprising; none of the fungi showed detectable clearing of a cellulose medium. They stated that despite repeated attempts with a wide selection of organisms, substrates, and cultural conditions, they were unable to isolate from the brown-rot fungi a cellulolytic enzyme system active on native cellulose.

Nitrogen utilization studies have been carried out by Jennison, Newcomb, and Henderson (1955) on *F. cajanderi* (as *F. subroseus*). Using synthetic media and shake cultures, they found that most organic and inorganic nitrogen sources

supported good growth of the fungus. The best growth (in terms of oven-dry weight) was obtained with casein hydrolyzate, DL-alpha alanine, glutamine, and DL-valine. Slightly less growth resulted with L-arginine HCl, L-asparagine, DL-aspartic acid, L-glutamic acid, glycine, and L-proline. Thiamine was required for growth and the optimum pH for the fungus was 4.2 using their synthetic media.

Jennison and Perritt (1960) reported that the D-forms of amino acids were not utilized by *F. cajanderi* (as *F. subroseus*). The DL-forms of amino acids showed only half the growth as the L-forms of amino acids at comparable concentrations. No carbon or vitamin utilization studies for *F. cajanderi* have been found in the literature.

MATERIALS AND METHODS

A. Development of mycelium and basidiocarps in nature

The development of *F. cajanderi* in nature was studied by collecting basidiocarps at various stages of maturity from different localities and substrata. In the laboratory, the specimens were analyzed and the developmental morphology followed from the decayed wood stage to the mature basidiocarp. The processing and storage of the collected material and the methods used for basidiocarp analysis are described under the headings: materials; embedding, sectioning, and staining techniques; hyphal analysis techniques; photographs.

1. Materials

Specimens of *Fomes cajanderi* were collected from different localities in central and southwestern Alberta and from northern British Columbia (see Appendix I for field data). Basidiocarps at various stages of development were collected along with some of the wood upon which they were growing. The type, condition, and exposure of the substrate was recorded and the presence of other visible fungi on the same substrate was also noted.

The basidiocarps were brought back to the laboratory in clean polyethylene bags sealed with elastic bands. Occasionally, clean paper bags were used, especially if the specimens could not be brought back to the laboratory within

a week. Collections left in polyethylene bags for longer periods sometimes showed undesirable renewed growth which often covered the pileus and pore surfaces with vegetative mycelium. Occasionally, the wood and basidiocarps would be contaminated by *Penicillium* and *Trichoderma*. In the laboratory, the collections were stored at 0-5°C or frozen for later examination and identification. Specimens stored at 0-5°C were examined macroscopically and microscopically within 1-3 days after collection. Cultures of dikaryotic mycelium were made at this time by removing bits of basidiocarp tissue or small slivers of infected wood with alcohol-flamed forceps and placing the material on plates of malt extract agar (Nobles, 1948).

Small collections, after being identified and/ or cultured, were dried for 1-2 weeks in a warm air cabinet and later stored in a herbarium in sealed polyethylene bags with a few moth crystals. If a collection contained many basidiocarps, the specimens were divided into two groups. One group was dried for herbarium purposes, the other group was stored frozen. The frozen material remained viable for a number of months and could be examined by dissection and maceration techniques or by embedding and sectioning. Cultures could be obtained from the basidiocarps or the infected wood immediately beneath the basidiocarps.

Both *F. cajanderi* and the closely related *Fomes roseus* grow in Alberta, although the latter species is less commonly found. Since *F. roseus* resembles *F. cajanderi* in

color, occasionally in form, and in preference for coniferous wood, it was imperative that positive identifications be made. Mounce and Macrae (1937) have shown that *F. roseus* and *F. cajanderi* can be positively distinguished by the shape and size of their basidiospores. In my study, the majority of collections were identified by the shape and size of natural or cultural spores. The basidiospores formed in culture were indistinguishable from those formed in nature. Since *F. roseus* and *F. cajanderi* frequently fruit in culture, spore examinations could easily be done. In the few instances where natural or cultural basidiospores could not be obtained, specimens were identified on the basis of pileus form and context color (Overholts, 1953) and by various cultural features (Nobles, 1958, 1965).

In addition to my own collections, dried specimens of *F. cajanderi* were examined from the Mycological Herbarium at Ottawa and the Cryptogamic Herbarium at the University of Alberta.

2. Embedding, sectioning, and staining techniques

Two methods were used to examine the vegetative mycelium in the wood. In the first method, temporary slides were prepared by hand-cutting thin sections of the infected wood with a razor blade and staining with 1% aqueous β-phloxine or cotton blue in lactophenol. This method was rapid and simple to perform, however, hyphae were sometimes difficult to observe in the wood. In the second and more satisfactory method, permanent slides were

prepared by cutting out small blocks of infected wood (approximately 1 in x 1/2 in x 1/2 in) and soaking them in distilled water overnight. The wood was usually sufficiently softened to allow sectioning at 15-20 μ increments with a sliding microtome (American Optical Instrument Co.). The wood sections were stained following a modified procedure of Cartwright (1929) (see Appendix II). The sections were stained in safranin and gently heated with picro-anilin blue over an alcohol lamp. After dehydration in an ethanol series (50%, 70%, 85%, 98%), the sections were cleared in clove oil, washed twice in xylene, and permanently mounted in Kleermount (Carolina Biological Supply Co.). Transverse, tangential, and radial sections were cut, with the latter two sections yielding better results.

Frozen basidiocarps (thawed at room temperature before use) at various stages of development and infected wood bearing primordia were examined using the paraffin technique of Johansen (1940). The material was first trimmed down to suitable size and then vacuum-infiltrated with weak chrom-acetic acid and left to fix for 24-48 hr. After washing in running water overnight, the material was dehydrated in an ethanol series (5% through to 30%) followed by an ethanol-tertiary-butyl alcohol series (50% through to 100% TBA). Tissuemat (Fisher Scientific Co.) was used as the embedding material. Sections were cut at 10-15 μ increments on a rotary microtome (American Optical Instrument Co.) and the ribbons flattened out on a warm slide flooded with 4%

formalin. Haupt's adhesive was used to mount the sections. A safranin-fast green staining schedule (Johansen, 1940) was followed and the sections later dehydrated. Permanent mounts were prepared by covering the sections with a drop of Kleermount and adding a coverslip. The coverslips were weighted down with lead weights while the slides were drying on a warming tray (40°C). Sections of the basidiocarps were made longitudinally along their length and width and transversely from the top of the pileus downward to the pore region in a horizontal plane. The embedded sections were useful in showing the organization and arrangement of hyphae in the developing basidiocarps. To examine long, intact lengths of hyphae dissection and maceration techniques were used for hyphal analysis.

3. Hyphal analysis techniques

Fresh, frozen, and dried specimens of *F. cajanderi* were used in the hyphal analysis. Frozen material was thawed before use and dried specimens were soaked in distilled water for several minutes to soften the material. Dissection of the basidiocarps involved teasing apart small pieces of tissue (approximately 2 mm x 1 mm x 1 mm) with dissecting needles in which the points were filed down to a fine, sharp edge (Teixeira, 1962). Pieces were cut from the pileus surface, context, margin, and pore regions of the basidiocarp with a razor blade and placed in 5% aqueous KOH which swelled the hyphae to their normal size. The

individual hyphae were teased out under X35 magnification using a Cooke, Troughton, and Simms stereomicroscope.

Similar sized pieces of tissue were also soaked in KOH and macerated by gentle tapping with a blunt glass rod to separate the hyphae. This method was much less tedious and time-consuming than the dissecting technique, but shorter lengths of hyphae were generally obtained. The dissecting and macerating techniques were necessary to observe the development of various hyphal types in the basidiocarps, since embedding and sectioning produced relatively short lengths of hyphae. The separated hyphae were stained with 1% aqueous β-phloxine or cotton blue in lactophenol.

4. Photographs

All photographs in this study were taken with the following equipment. Micrographs were taken with a Pentax 35 mm camera mounted on a Leitz microscope and on a Cooke, Troughton, and Simms stereomicroscope. A Vickers Photomicro 35 mm camera fitted to a Vickers photomicroscope was also used. The macroscopic photographs were taken with a Pentax 35 mm camera and a set of close-up lenses.

B. Development of mycelium and basidiocarps in culture

The growth of the vegetative mycelium and basidiocarp development was studied with three types of cultures: agar culture, still (liquid) culture, and wood-block culture. In addition, three physiological aspects of mycelial growth

were examined which included growth rates at various temperatures in agar culture, carbon utilization in shake culture, and growth in wood-shaving cultures supplemented with L-asparagine. The cultural studies of *F. cajanderi* emphasized the developmental morphology of basidiocarps, since this polypore species readily fruited in culture. By studying the basidiocarps formed in culture, a better understanding of the hyphal basis of development in the natural basidiocarps can be obtained.

1. Development in agar culture

During routine culturing of *F. cajanderi* collections, it was noted that many isolates readily fruited on Nobles' malt agar (Nobles, 1948). Because of the good growth and consistent fruiting on this medium and since the cultural methods described by Nobles (1965) were followed, Nobles' malt agar (see Appendix III) was used as the standard agar medium in the cultural studies.

The sources of the stock cultures were the dikaryotic vegetative mycelium from various wood substrata and the dikaryotic mycelium of natural basidiocarps. Small pieces of decayed wood or context tissue were removed from collections with flame-sterilized forceps and partially embedded in Nobles' malt agar. Pure cultures were obtained by sub-culturing from these isolation plates. Stock cultures were maintained at 4-5°C and grown in tubes containing 6 ml of Nobles' malt agar.

Cultures to be studied for mycelial and basidiocarp

development were started by transferring pieces of dikaryotic mycelium from stock tubes to Petri plates of malt agar. The plates were left at room temperature (23 ± 1.5°C) for 7-10 days. From the colony edges of the source inoculum plates uniform 7 mm mycelial plugs were cut out with an alcohol-flamed cork borer. The inoculum plugs were placed mycelium side up in Petri plates containing malt agar. The plates were either the glass type, 9.0 cm in diameter, or the pre-sterilized 8.5 cm plastic, disposable type. It was found that the agar in the plastic Petri plates dried out relatively rapidly if the plates were not kept in polyethylene bags during incubation. Thus cultures growing in plastic Petri plates were incubated in polyethylene bags sealed with elastic bands. Since the agar medium in the glass Petri plates did not dry out as readily as the agar in the plastic plates, these plates were usually not placed in polyethylene bags during incubation.

The inoculated plates were placed near a south-facing window or in a dark cupboard or incubation chamber under conditions of ambient room temperature and relative humidity. A sufficient number of plates was inoculated so that periodically plates could be removed and the fruiting structures examined and described. All agar cultures were kept for at least 8 weeks before being discarded.

Because basidiospores often formed in culture and were used for identification, all plates were stored upside down so that spores could be deposited on the lids. The

spore color could thus be determined and spores could be scraped off the lid with a flame-sterilized dissecting needle and mounted in distilled water or 5% aqueous KOH for examination.

The fruiting structures formed in agar culture were examined by hand-sectioning the poroid areas with a razor blade or by dissecting out the hyphae using techniques described for the natural basidiocarps. The hyphae and sections were mounted in 5% aqueous KOH and stained with 1% aqueous β-phloxine or cotton blue in lactophenol.

2. Development in still culture

A 1% malt extract liquid medium was used throughout the studies of fruiting development in still culture. The procedure was to inoculate 250 ml Erlenmeyer flasks containing 50 ml of 1% (w/v) malt extract (Difco Laboratories) broth with 7 mm mycelial plugs. The inoculum plugs were cut from 7-10 day-old agar cultures of *F. cajanderi*. Each flask received one mycelial plug. The flasks had been machine-washed with Merit PLX detergent (Economics Laboratories, Toronto) and rinsed 3 times with tap water and once with distilled water. They were dried in a hot air oven for 1 hr.

The flasks were stoppered with synthetic sponge plugs which were covered loosely with aluminum foil to prevent excess evaporation of the medium. They were placed on a laboratory bench under conditions of ambient room temperature, light, and relative humidity. At periodic intervals,

a number of flasks was examined and the vegetative mycelium and fruiting structures described. The still cultures were kept for at least 4 months before being discarded. The same examination techniques described for the agar cultures were used.

3. Development in wood-block culture

A wood-block culture experiment was set up to compare the fruiting development of *F. cajanderi* on this substrate with that of malt agar and still cultures. Isolates B-3, B-128, and B-189 were studied. The three isolates were chosen because they fruited consistently on malt agar.

Fourteen blocks each of poplar, spruce, and pine, measuring approximately 3 cm x 1.5 cm x 1.0-1.5 cm, were cut from sound sapwood and soaked for 48 hours in sterile, distilled water. The wood blocks were then removed from the water and sterilized in empty deep culture dishes, 10.0 cm x 8.0 cm, at 121°C for 20 min at 15 lb pressure. After sterilization, the blocks were transferred aseptically to 9 day-old cultures of *F. cajanderi* growing in deep culture dishes that contained approximately 85 ml of malt agar. Each culture dish received one wood block. There were 12 wood-block cultures of each isolate, with 4 replicate cultures each of poplar, spruce, and pine. Six contamination controls were also set up, with 2 blocks of each tree species.

Half of the wood-block cultures were placed in an incubator at 20°C with an alternating 12 hr light and dark

cycle. During the last 2 months of incubation the temperature was reduced to 15°C. The other half of the wood-block cultures were placed on a laboratory bench at ambient room temperature and under laboratory light conditions. All cultures were stored in sealed polyethylene bags to minimize contamination and reduce excess desiccation of the cultures. At the end of 12 months, the vegetative mycelium and the fruiting structures were examined and the wood-blocks sectioned using the techniques described for natural material.

4. Temperature and growth of vegetative mycelium

The growth rate of three isolates of *F. cajanderi* at different temperatures was examined by measuring linear growth on an agar medium. The isolates (B-3, B-128, and B-189) were grown at the following temperatures: 11°, 15°, 20°, 25°, 28°, 32°, and 37°C.

Mycelium from stock slants were transferred to plates of malt agar and left for 7 days at room temperature and under ambient laboratory light. From the edge of these source inoculum plates, 7 mm mycelial plugs were cut out with a sterilized cork borer. The inoculum plugs were placed mycelium side up at the edge of 8.5 cm plastic, disposable Petri plates containing approximately 20 ml of malt agar. There were 5 replicate plates per isolate for each temperature. The inoculated plates were stored upside down in sealed polyethylene bags and incubated in the dark. The only light received by the cultures was during the

periodic measurements. No attempt was made to control the humidity of the incubation chambers.

Four days after the plates were inoculated, the first measurements were taken of the radial growth from the edge of the inoculum plug to the colony margin. Thereafter, the measurements were taken every 2 days for 14 days. When measuring, the plates were held up to a microscope lamp to allow the colony margin to be more readily distinguished. Two measurements were taken of each plate and the distances averaged. The results in Figure 47 (page 110) are the average of 5 plates per isolate for each temperature and are given as daily radial growth.

5. Growth-curve and carbon utilization studies

The method involved the determination of dry weight of dikaryotic mycelium grown in shake culture with various media. Stock cultures of *F. cajanderi* (isolate B-3) were maintained on slants of malt agar and stored at 4-5°C.

When an experiment was started, 7 mm mycelial plugs were cut with an alcohol-flamed cork borer from the edge of a 7 day-old malt agar culture grown at room temperature (23 ± 1.5°C). The inoculum plugs were transferred to 250 ml Erlenmeyer flasks containing 50 ml of 1% (w/v) malt extract medium. Each flask received one inoculum plug. All flasks used in the experiments were stoppered with synthetic sponge plugs which were covered loosely with aluminum foil to reduce evaporation. The flasks were placed on a rotary shaker (Lab-line Instruments) set at 185 oscillations per

minute with a diameter of motion of 1 inch. The same rotary shaker was used in all shake culture experiments. The malt extract flasks were incubated on the shaker for 15 days under conditions of ambient room temperature and laboratory light.

A standard, aseptic procedure was then followed to produce the fragmented mycelium to be used for inoculating the flasks. The medium of one of the source inoculum flasks was decanted off leaving the mycelial pellet. Fifty ml of sterile, distilled water were added to the flask and the contents briefly swirled. The water was then decanted off and another 50 ml aliquot of sterile, distilled water was added. The washing was repeated twice more so the mycelial pellet was washed three times in all. The washed mycelial pellet was then placed in a sterile Waring blendor with 150 ml of sterile, distilled water and homogenized at medium speed for 3 min. The blendor had been previously washed with Organisol detergent (Fisher Scientific Co.) and rinsed 6 times with tap water and 6 times with distilled water. Sterilization involved rinsing the blendor with 200 ml of 50% sodium hypochlorite ("Perfex") (Ward and Colotelo, 1960). The blendor was then rinsed at least 6 times with sterile, distilled water.

After homogenation, a 100 ml aliquot of mycelial suspension was removed and placed in a sterile 500 ml Erlenmeyer flask containing a Teflon coated stirring rod. Three 5 ml samples were removed and the flask was placed in

a 4-5°C refrigerator. The three samples were filtered separately on previously dried and tared Whatman No. 1 filter papers (11.0 cm diameter) using a Buchner funnel and a vacuum pump (Millipore Filter Co.). The papers were dried for 6 hr at 90-95°C. They were then placed in a desiccator (containing anhydrous calcium chloride) for 5 min to cool down and weighed immediately on a Mettler analytical balance to the nearest mg. In all experiments the concentration of the mycelial suspension was approximately 0.2 mg dried mycelium per ml and this concentration was left unadjusted. One ml of mycelial suspension was used to inoculate each flask.

During the inoculations, the flask containing the mycelial suspension and stirring rod was placed on a magnetic stirrer set at low speed. This was suggested by Dr. Colotelo to ensure that the mycelium remained suspended so a more uniform inoculum mass was dispensed. A sterilized, large bore 5 ml pipette was used to dispense the inoculum to each flask. For each experiment, three samples of the mycelial suspension were plated on malt agar to check for contamination. None of the control plates showed contamination after a 2 week incubation period.

A synthetic medium modified from Jennison, Newcomb, and Henderson (1955) was used in the carbon utilization experiments (see Appendix III). The carbon, nitrogen, and salt components of the medium were autoclaved separately and then aseptically added together after they had cooled.

The various components were autoclaved at 121°C for 15 min at 15-18 lb pressure. The thiamine component was filter-sterilized using Millipore Type HA filters (0.45 µ pore size). The medium was dispensed to each flask with a 10 ml capacity automatic syringe that had been autoclaved previously.

The cultures were grown in 250 ml Erlenmeyer flasks containing 50 ml of medium. They were closed with synthetic sponge plugs which were loosely covered by aluminum foil. The flasks used in all experiments were machine-washed with Merit PLX detergent and rinsed 3 times with tap water and once with distilled water. The flasks were then soaked in a dichromate-sulphuric acid cleaning solution for 1 hr and rinsed 6 times with tap water and twice with distilled water. They were dried in a hot air oven for 1 hr.

The carbon sources examined were dextrose, galactose, D-xylose, D-mannose, D-fructose, sucrose, maltose, and cellobiose. Whatman cellulose powder was tried, but it was not soluble in the synthetic medium used. The effect of carry-over of carbohydrates by the inoculum mycelium was tested with negative controls. In these control flasks, the carbon component of the medium was replaced by an equal volume of distilled water. A number of contamination controls were also set up. For each carbon source, 1-2 flasks without inoculum were incubated with the other flasks.

All carbon utilization experiments had an incubation

period of 23 days. The incubation time was determined by preliminary growth-curve experiments. The flasks were incubated on a rotary shaker and left at room temperature and under ambient laboratory light. At the end of the 23 day incubation period the mycelial pellets were harvested by filtering on dried, tared Whatman No. 1 filter papers using a Buchner funnel and a vacuum pump. The pellets were washed with 200 ml of distilled water to remove any excess medium. The filter papers were dried in hot air ovens set at 90-95°C for 22-24 hr. Prior to weighing, the filter papers were placed in a desiccator for 5 min to cool down and then weighed immediately on a Mettler analytical balance to the nearest mg. The pH of the culture filtrate of each flask was measured immediately after harvesting with a Radiometer (model 29) pH meter.

In the growth-curve studies two shake culture experiments were set up using isolate B-3. The first experiment involved following the growth of mycelium in 1% (w/v) malt extract medium at room temperature. The mycelium was grown in 250 ml Erlenmeyer flasks containing 50 ml of medium. The same inoculating and harvesting procedures were used as previously described. At 7 day intervals 6 flasks were harvested for a period of 5 weeks and the growth measured as mg dry weight of mycelium per flask. In the second experiment the growth in dextrose-salts medium was followed. The composition of the medium was the same as the dextrose-salts medium used in the

carbon utilization experiments. The inoculating and harvesting procedures were also the same. At 4 day intervals 5 flasks were harvested for a period of 36 days. Growth was measured as mg dry weight of mycelium per flask. In both experiments the pH of the culture filtrate of each flask was measured immediately after harvesting.

6. Growth on wood-shavings with L-asparagine

The basic procedure followed was adapted from Schmitz and Kaufert (1936). Wood-shavings were cut from a sound board of white spruce sapwood with a power planer. The shavings were spread out in a thin layer on clean laboratory benches (covered with paper) and air-dried for 8 days at room temperature. They were periodically mixed to ensure even drying. After drying, 25.00 gm samples of wood-shavings were weighed out on a Mettler analytical balance and packed into previously weighed deep culture dishes (with lids) measuring 10.0 cm x 8.0 cm. Ten culture dishes containing wood-shavings were then chosen at random and dried at 90-95°C until they reached a constant weight. The oven-dry weights of the ten samples were averaged and their value was used to calculate the oven-dry weight of the remaining samples of air-dried wood-shavings.

Stock solutions of L-asparagine (Nutritional Biochemical Corp.) at concentrations of 0.1%, 0.5%, 1.0%, and 2.0% (w/v) were prepared in distilled water. L-asparagine was chosen as the nitrogen source since Jennison, Newcomb, and Henderson (1955) had reported that this amino acid supported

good growth of *F. cajanderi* in a synthetic medium. To each culture dish 50 ml of asparagine solution were added giving the wood-shavings a moisture content of approximately 200 percent.

The culture dishes and their contents were sterilized by autoclaving at 121°C for 20 min at 16 lb pressure. After the culture dishes had cooled, they were inoculated with one mycelial block (approximately 5 mm^2) cut from the edge of 12 day-old cultures growing on malt agar. Two isolates of *F. cajanderi* (B-3 and B-128) were investigated. For each isolate there were 5 replicate cultures per nitrogen concentration. Five replicates of each isolate were set up as negative controls in which an equal volume of distilled water was added instead of the asparagine solution. There were 3 culture dishes of each nitrogen concentration left uninoculated to serve as contamination controls.

The culture dishes were placed in polyethylene bags sealed with elastic bands and incubated in a dark cupboard at room temperature (23 ± 1.5°C). Storage in the bags minimized contamination and reduced desiccation of the cultures. At the end of 12 months, the culture dishes and their contents were oven-dried to a constant weight at 90-95°C and the loss of weight due to decay was determined. The weight of the L-asparagine and inoculum blocks was accounted for in the calculations.

OBSERVATIONS

A. Development of mycelium and basidiocarps in nature

1. Growth of the vegetative mycelium

The appearance of the vegetative mycelium is similar in the naturally-infected wood and the artificially-infected wood in the early stages of decay. Since *F. cajanderi* is found mainly on coniferous wood, the following observations will describe the appearance of the mycelium in spruce and pine wood. In the early stages of decay the mycelium is sparse and grows in a sinuous manner through the lumina of the tracheids, primarily in a longitudinal direction (Figure 1). In all observations of the infected wood, only the dikaryotic, clamped type of mycelium was seen. The hyphae are hyaline, thin-walled, 0.9-1.8 μ in diameter, and often branched. Branching generally occurs near the ray cells where the hyphae pass from one tracheid to the next. The hyphae also penetrate the tracheids via the bordered pits (Figure 2). As the hyphae pass through the ray cells or pits branching may occur. In radial and tangential sections it is readily apparent that the mycelium is found most abundantly near the ray cells. In other areas of the tracheids the mycelium is very sparse with only one or two hyphae passing through a tracheid lumen.

In the advanced stages of decay the mycelium is found in dense aggregations in the tracheids near the ray cells

(Figure 3). The hyphae in these aggregations are hyaline, 0.9-2.6 μ in diameter, thin-walled, and clamped. Typical clamp connections are predominant, but very infrequently "medallion" clamps are observed. These clamp connections were first observed by Falck (in States, 1969, p. 53) who named them "medallion" or "handle" clamps because of the large space between the clamp and the parent hypha. The "medallion" clamps are distinctive in the genus *Gloeophyllum* (*Lenzites*) where they were first noted. The presence of "medallion" clamps on the vegetative mycelium of *F. cajanderi* has not been previously described. The hyphae in the aggregations are profusely branched and are intertangled and interwoven. At this stage of decay, the tracheids are weakened and perforated with bore holes. The wood is soft and tears easily when sectioned. It is thought the bore holes are formed by exogenous enzymes released by the infecting hyphae as they pass through the tracheid walls. The diameter of the bore holes is much larger than that of the penetrating hyphae. No constriction of the hyphae is observed as they pass through the bore holes. In rare instances, bore holes exhibiting a cracked appearance are seen. The edges of these bore holes are broken in 3-4 places with the cracks radiating outwards from the hole. The same form of bore hole has been reported by Hubert (1924) who believed the cracks developed because of shrinkage of the wood.

The dense hyphal aggregations are abundant near the

wood surface. In some tracheids the hyphal aggregations are so dense that the lumina are completely filled. The tracheid walls are perforated by numerous bore holes in all areas. The wood itself is brown-colored and cracked into cubes (Figure 5). These cubes are often dry to the touch and crumble easily into a brown powder. Pockets of cubical rot have not been observed in the wood as some earlier workers have reported. The brown rot caused by *F. cajanderi* occurs throughout the sapwood and heartwood areas of the wood, although the initial stages of decay are confined to the heartwood.

In the advanced stages of decay the mycelium near the wood surface emerges through cracks and fissures in the wood or bark (Figure 25). The emerging hyphae have thick, brown walls and are occasionally branched. They range in diameter from 0.9-3.5 μ and are aseptate. The tracheids may be completely filled by dense masses of these thick-walled hyphae (Figure 6). The hyphae emerging from the wood form the basidiocarp initial or primordium. The morphology and formation of this structure will be described in the section on primordial development.

In wood that has not yet broken up into cubical form, red to reddish-brown stained areas often develop. These stained areas are either thin, sharp lines (Figure 7) or broad, wide areas with irregular edges (Figure 8). The tracheids at the edges of the stained areas are filled with dense hyphal aggregations composed of dark-brown, thick-

walled hyphae (Figure 9). The walls of the tracheids are reddish-brown and occasionally, a dark-brown, amorphous substance is seen among the hyphae. The identity of this substance is not known. The stained appearance of the wood is due to the dark-brown hyphal aggregations in the tracheids and the reddish-brown color of the tracheid walls. It is possible that substances are released by the hyphae that cause the staining of the tracheid walls.

In the deep cracks of heavily-decayed wood under moist, shaded conditions, mats of light-pink to white mycelium occasionally form. The mycelium is composed of long, flexuous, thick-walled, aseptate, refractive hyphae, 0.9-3.5 μ in diameter. These hyphae are sparingly branched and loosely intertangled and interwoven to form a cottony to cottony-floccose mat that separates easily from the wood surface. Among the thick-walled hyphae are thin-walled, hyaline, clamped hyphae that are 0.9-2.6 μ in diameter and frequently branched. These thin-walled hyphae are intertangled among the thick-walled hyphae in an unorganized manner. The light-pink color of the mat appears to be due to the light yellowish-brown walls of the thick-walled hyphae. These hyphae are light-pink colored when observed collectively.

2. Development of the basidiocarp

During the course of investigating the development of natural basidiocarps, Corner's papers (1932a, 1932b, 1953) were especially useful in describing the hyphal types

observed. The terminology used by Corner is of a descriptive character and the following outline of terms is based on his 1953 paper. Corner noted that polypore basidiocarps were composed of three types of hyphae which he named generative, skeletal, and binding. The skeletal hyphae are "unbranched, thick-walled, commonly aseptate, longitudinal, constructional hyphae of the first order in the growing region." Binding hyphae are "much branched, narrow, rarely septate, thick-walled hyphae of very intricate and limited growth, developing behind the growing point." The generative hyphae are "thin-walled, branching and septate hyphae which take a longitudinal course in the growing region." These thin-walled hyphae usually remain thin-walled or they may become thick-walled. The generative hyphae produce "the system of skeletal hyphae, which is the framework, and the system of binding hyphae, which are the ties." Polypore basidiocarps consist of various combinations of the three hyphal types. The basidiocarps may be monomitic, dimitic, or trimitic in construction depending on the hyphal types present. Monomitic basidiocarps consist entirely of generative hyphae, dimitic basidiocarps consist of either generative and skeletal hyphae or generative and binding hyphae, and trimitic basidiocarps consist of all three types of hyphae. The results of the basidiocarp analysis of *F. cajanderi* revealed that this polypore is dimitic with generative and skeletal hyphae being present. The skeletal hyphae observed agreed essentially with Corner's definition

except that branching occurred sparingly in the apical portions of the hyphae.

a. Macrostructure of the basidiocarp

Typical basidiocarps of *F. cajanderi* are sessile (Figure 10) or effused-reflexed (Figure 11). The pileus form varies from applanate (Figure 12) to convex (Figure 13) and is frequently dimidiate. Young basidiocarps may be somewhat ungulate in form. The convex form is often conchate as seen in cross-section in Figure 19. The resupinate form of basidiocarp has been observed in one collection (B-333) where it measured 17.5 x 7.3 cm (Figure 15) and showed no evidence of pileus formation. Basidiocarps developing along the lower portions of a log may be temporarily resupinate in form (Figure 22), but when fully developed they become effused-reflexed. The fresh, moist basidiocarps of *F. cajanderi* are leathery to corky in consistency, but when dry they become subflexible to rigid. The number of basidiocarps developing on a log or stump is variable. In some instances, over 20 basidiocarps have been observed growing in close proximity on a single log, but the usual number observed is 5-10 basidiocarps. It is often difficult to distinguish between individual basidiocarps, since they may fuse laterally and become confluent (Figures 10, 11). Basidiocarps frequently overlap each other and form an imbricate structure (Figure 14). The basidiocarps develop on the barked and debarked portions of the wood. Barked wood generally bears a greater number

of basidiocarps, possibly due to the retention of favorable moisture levels in the wood by the bark. The basidiocarps develop from primordia that emerge through cracks and fissures in the bark. Figure 16 shows four sessile basidiocarps that have formed on the barked portion of a log. On debarked logs the basidiocarps develop in the large, horizontal cracks in the wood or from broken branch stubs. Basidiocarps that develop in the large cracks are often laterally fused (Figure 17) and may reach lengths of 20 cm. Formation of the basidiocarps generally occurs along the sides of a log. In some instances, basidiocarps may develop along the lower portions of a log if it is raised off the ground. These basidiocarps become effused-reflexed in form. Occasionally, the basidiocarps will form at the top portion of a log emerging from a crack or broken branch stub. These basidiocarps usually become sessile in form. Infrequently, a circular and applanate basidiocarp develops (Figure 12). The ends of cut logs occasionally bear basidiocarps which are typically sessile in form.

The pileus surface of the basidiocarps is extremely variable in color depending on the stage of development and the effects of weathering. Initially, the pileus surface is light-pink to pinkish-red. Later the surface becomes light to dark reddish-brown or reddish. In old basidiocarps that have overwintered, the pileus surface is weathered to shades of grayish-brown to grayish-black or black. Basidiocarps that have formed in exposed, sunny

sites are often bleached to grayish-white. Zonation of the pileus surface is variable and may be azonate (Figures 10, 12) or zonate. The zones are due either to alternating light and dark bands of the pileus surface (Figures 13, 14) or to shallow furrows that form on the surface (Figure 18). Young basidiocarps have a finely and compactly tomentose pileus surface. In fully developed basidiocarps the surface becomes glabrous or radiately fibrillose (Figure 13). The tomentose surface may persist at the marginal regions (Figure 21) and in areas of renewed growth. The pileus surface can become hard and furrowed but it does not become incrusted at any stage.

The color of the pore surface varies from rosy-pink to pinkish-red in newly formed pores to reddish-brown in the older pore areas. The pore surface is even and plane in sessile basidiocarps but more irregular and hummocky in effused-reflexed basidiocarps. The pore mouths are circular to sub-angular in shape, thick, entire, and average 4-6 per mm. In young basidiocarps the pore mouths tend to be round and very thick-walled (Figure 22), but in fully developed basidiocarps the pore mouths are sub-angular and the pore walls thinner (Figure 20). The depth of the pores range from 0.5-2.0 mm for each growing season. The pores are shallowest at the edge of the marginal region and in young basidiocarps (Figure 24). Stratification is rarely observed in the pores. The older pores are often filled with hyphae giving a "white, stuffed" appearance (Figure

19). The pores of the effused-reflexed basidiocarps are frequently daedaloid in the effused portions of the pore surface (Figure 23). These pore mouths are elongated and the pore walls thin and somewhat wavy. A sterile lower margin is present at all stages of basidiocarp development. This lower margin is 0.5-2 mm wide and reaches its greatest width in young basidiocarps (Figure 22). The outer edge of the margin in young basidiocarps is broad and rounded while in fully developed basidiocarps the margin edge is thin and acute.

The color of the context is variable and ranges from rosy-pink in the marginal regions to pinkish-brown, reddish-brown, or brown in the older regions. As Overholts (1953) has pointed out, the darker tinted context of *F. cajanderi* is a character useful in distinguishing this species from *F. roseus* which has a silvery-pink context. The thickness of the context ranges from 1-12 mm. It is thinnest at the margin and gradually thickens when followed back from the margin edge. Measurements of the context thickness were taken two-thirds of the distance from the margin edge to the substrate. Zonation is often observed in the context (Figure 24). These zones are thought to represent periodic spurts of radiate growth that occur during favorable growing conditions. The edges of the zones are darker colored where growth has been halted temporarily. The firmness of the context ranges from soft and punky in the marginal regions to hard-corky in the older regions. When the

context is touched with a drop of 5% aqueous KOH solution, the tissue immediately turns black.

Collections of *F. cajanderi* have been found growing saprophytically, usually on conifer logs and stumps. A list of the collections of the fungi examined from various substrates is given in Table 1. In Alberta the great majority of specimens were collected on conifers, particularly *Picea*. A number of collections have been found on *Pinus* and two collections were noted on *Populus*. Fifteen collections were examined from unclassified conifer wood in which the substrate could not be identified. Specimens examined from Ottawa were collected from a variety of substrates. They included *Picea*, *Pinus*, *Populus*, *Larix*, *Abies*, *Pseudotsuga*, *Thuja*, *Tsuga*, and *Prunus*. The Ottawa specimen growing on *Populus* (DAOM 52060) produced a typical brown cubical rot of the sapwood (Figure 4). Basidiocarps of *F. cajanderi* grow most abundantly in fairly moist conditions, usually on barked logs that are partly shaded. When collected from exposed, debarked logs the basidiocarps are typically smaller and fewer in number. The pileus surface is also lighter colored in the drier sites.

A general description of the macroscopic features of *F. cajanderi* basidiocarps examined is given below.

Basidiocarps sessile to effused-reflexed, occasionally resupinate when young but later becoming effused-reflexed; consistency when fresh leathery to corky, when dry subflexible to rigid; pileus applanate to convex, dimidiate,

Table 1. Collections of *F. cajanderi* examined from various substrates.

Substrate	U. of A. Herbarium	Research Collections	DAOM*
Picea	19	38	1
Pinus	8	5	1
Populus	0	2	1
Larix	0	0	2
Abies	0	0	1
Pseudotsuga	0	0	1
Thuja	0	0	1
Tsuga	0	0	1
Prunus	0	0	1
Conifer wood (unclassified)	7	8	0

* Mycological Herbarium at Ottawa.

somewhat ungulate when young; size 0.5-3.5 x 0.8-12.5 x 0.3-2.4 cm, when laterally fused reaching 20 cm in length; pileus surface color initially light-pink to pinkish-red, later light to dark reddish-brown or reddish, becoming grayish-white, grayish-brown or black, surface at first finely and compactly tomentose, especially at marginal regions and areas of renewed growth, gradually becoming glabrous or radiately-fibrillose, surface azonate or zonate, zones due to light and dark colored bands or shallow furrows, no incrustation at any stage; margin broad and rounded when young, later becoming thin and acute, lower sterile margin always present, 0.5-2 mm wide; pore surface rosy-pink to pinkish-red at first, later reddish-brown, surface even and plane to irregular and hummocky, pore mouths round and thick-walled when young, sub-angular and thinner-walled when fully developed, pores of effused-reflexed basidiocarps daedaloid on effused portions, pores average 4-6 per mm, pore depth 0.5-2 mm per growing season, stratification rarely observed, older pores often "white, stuffed"; context rosy-pink, pinkish-brown in marginal regions, reddish-brown to brown in older regions, context 1.0-12 mm thick, often zonate, firmness of context soft, punky to hard-corky, context turns immediately black when touched with KOH.

b. The primordium

The dense aggregations of mycelium in the tracheids give rise to the basidiocarp initial or primordium that

forms at the surface of the wood or on the bark. The primordium is considered here to be the first stage of basidiocarp development. During this stage the mycelial mass emerges from the wood and develops into a compact structure, but has not differentiated to form a pore surface. The mycelium in the wood emerges through a crack in the wood or bark (Figure 25) and grows outward in a radial manner. The size of the primordium is variable, from 2-9 mm across and 1.5-4 mm in thickness. The primordium is hemispherical in shape (Figure 26) and light to dark reddish-pink colored when it first emerges from the wood. Later, the color becomes reddish-brown to medium-brown. The surface is tomentose to glabrous in character. The transition from the vegetative mycelium in the wood to the generative and skeletal hyphae in the primordium is not distinct. In the tracheids immediately below the primordium dense aggregations of brown-colored, aseptate, thick-walled hyphae and thin-walled, hyaline, clamped hyphae are observed. The outward growth of hyphae from these aggregations through cracks in the wood or bark results in the formation of the basidiocarp primordium.

The hyphae in the primordium are not in strict parallel alignment but they do grow outward in a radial manner (Figure 27). Two types of hyphae are found in the primordium: the generative hyphae and the skeletal hyphae. The generative hyphae are granular appearing, 1.7-3.3 μ in diameter, occasionally branched, and have clamp

connections at each septum. The walls are thin and hyaline. The skeletal hyphae, which are the predominant hyphae in the primordium, are refractive, aseptate, 2.2-5.5 μ in diameter, and thick-walled. The lumina are narrow, lightly stained with phloxine, and nearly occluded by the hyphal walls which are 0.8-1.1 μ thick. The proximal and apical portions of the skeletal hyphae are thinner in wall thickness and the lumina are wider. Branching is sparse on these hyphae. The reddish-pink color of the primordium appears to originate in the walls of the skeletal hyphae. These walls are yellowish-brown colored and refractive (shiny-appearing) when observed microscopically. The reddish-pink color is apparent when the skeletal hyphae are observed collectively.

The primordial stage is infrequently found in the field, since soon after emergence and formation pore development begins. The size of the primordium when pore development starts may be as small as 4-6 mm across and 2-4 mm thick. The developing pileus surface is variable in color, from pinkish-red to dark reddish-brown. The surface may be glabrous or finely tomentose and rugulose (Figure 28). The developing pore surface is light-pink or pink. The young pores are thick-walled, very shallow in depth, circular in shape, and the marginal region is rounded and blunt.

c. Microstructure of the basidiocarp

In describing the microstructure of the basidiocarp four major regions will be examined. They are the margin,

the context, the pileus surface, and the dissepiment regions. The different types of hyphae found in these regions will be described as to their morphology and development. Figure 29 shows these regions in a vertical section of a *F. cajanderi* basidiocarp.

i. The marginal region

In the marginal region of the developing basidiocarp, up to between 2000 and 3000 μ back from the outer edge of the basidiocarp, there are two types of hyphae, the generative hyphae and the skeletal hyphae. The generative hyphae are abundantly clamped, thin-walled, occasionally to frequently branched, and 1.5-3.0 μ in diameter. The branches arise near or directly from the clamp connections (Figure 30). The generative hyphae are found throughout the marginal region, evenly distributed among the skeletal hyphae, and growing in the same direction. Occasionally, small "islands" of generative hyphae are observed in a dense mass among the skeletal hyphae.

The skeletal hyphae are aseptate, long, flexuous, infrequently branched, and 2.2-5.5 μ in diameter. Developing skeletal hyphae are frequently observed in the margin. Often the proximal end of these hyphae is still attached to the generative hypha by the clamp connection (Figure 31). The cytoplasm in the apical ends of the developing skeletal hyphae stains deeply with phloxine and appears granular. The hyphal walls are thin in the apical portions and may remain so for over 100 μ back from the growing apex. In

the very early stages of development skeletal hyphae may have thin walls throughout their entire length. These hyphae are usually less than 100 μ long. In developing skeletal hyphae, longer than 100 μ, the hyphal walls gradually thicken and become brown-colored and refractive as they are followed back from the apical end to the proximal end. As a skeletal hypha begins its growth from a generative hypha its diameter is the same as the generative hypha, but as growth continues the diameter gradually increases. A developing skeletal hypha may begin growth from the generative hypha with a diameter of 2.3 μ and eventually reach a diameter of 5.5 μ in the apical portions. The length of the developing skeletal hypha varies depending on the stage of development. In the marginal region lengths range from less than 100 μ to more than 740 μ. Occasionally, a developing skeletal hypha in the margin has two or three branches forming at the apical end. These branches are slightly narrower than the main hypha from which they arose. The growth form of the branches may be quite tortuous and contorted. The branches have no clamp connections or septa and their walls are thick, refractive, and brown-colored. Branch lengths have been measured up to 250 μ. In the marginal region the skeletal hyphae have all originated as terminal cells of the thin-walled generative hyphae. New skeletal hyphae are continually being formed from the generative hyphae as the margin grows outward in a radiate fashion. The skeletal hyphae are arranged in

parallel alignment in the margin which provides support to the generative hyphae and gives the basidiocarp rigidity and form (Figure 32).

ii. The context region

In the context region, beginning approximately 3000 μ back from the outer edge of the basidiocarp, the generative hyphae usually develop irregularly thickened walls and the lumen becomes narrow and partially occluded. The first indication of wall thickening is the swollen, solid appearance of the clamp connections. The swollen appearance of the clamp connections gives the impression of a ball-and-socket arrangement. Small, solid, and refractive protuberances will occasionally form along the hyphal walls. Eventually, most of the hyphal wall becomes irregularly thickened so that the lumen is seen as a narrow, sinuous line running from side to side in the hypha. The irregularly thickened hyphae are tortuous and contorted in form, 2.0-7.0 μ in diameter, and frequently branched (Figure 33). One feature of these hyphae is their marked tendency to separate at the clamp connections (Figure 34). There appears to be little solid attachment of the adjacent hyphal cells on either side of the clamp connection. The hyphae frequently break at the clamp connections leaving one hyphal cell with the hooked portion of the clamp. There is no definite orientation of the irregularly thickened generative hyphae in the context. They are usually densely massed together and intertangled with each other. These

hyphae are abundant throughout the context region and "islands" of them will be observed among the skeletal hyphae.

Thin-walled generative hyphae are infrequent in the context region as most of them appear to have developed into the irregularly thickened generative hyphae. The thin-walled hyphae are frequently branched, 1.5-3.0 μ in diameter, but more contorted and tortuous in form than those seen in the marginal region. The cells of these hyphae are occasionally seen devoid of contents and hyaline in appearance.

The skeletal hyphae in the context are long, flexuous, and arranged in a longitudinal direction radiately outward (Figure 36). Some skeletal hyphae interweave across the main direction of growth: these hyphae are contorted and tortuous in form. The skeletal hyphae are 1.7-5.5 μ in diameter, aseptate, with hyphal walls 0.8-1.1 μ thick. The lumen is narrow and appears granular, staining lightly with phloxine. The walls are refractive and light to dark yellowish-brown. The skeletal hyphae are the dominant hyphae in the context. They have developed in the marginal region from the thin-walled generative hyphae. As the margin continues to grow outwards, the developing skeletal hyphae mature. They develop thick, refractive walls and the lumen contents gradually disappear. The mature skeletal hyphae in the context provide the supporting elements of the basidiocarp. The majority of the skeletal hyphae are unbranched along their entire length which can

reach 1700 μ. Branches that do form may be found in the central portions of the hyphae or at the apical portions. Teixeira (1962) has referred to skeletal hyphae that form branches at the ends as "arboriform" hyphae because of their tree-like appearance. Figure 35 shows the branched end of one such skeletal hypha. The diameter of the branches is slightly narrower than the main hyphae. The branches at the apical portions are not in parallel alignment, but weave in and out among the other skeletal hyphae. Due to the restricted growth among the skeletal hyphae, the growth form of the branches may become quite tortuous. The number of branches formed on a particular skeletal hypha can vary from one to five. In some instances the branching may be dichotomous, but this occurs infrequently. The contorted and tortuous branches of the skeletal hyphae may function as binding elements that serve to hold together the other hyphae in a compact, firm mass.

iii. The pileus surface

The pileus surface of the marginal regions in the developing basidiocarps and in basidiocarps where renewed growth has occurred (Figure 29) is reddish-pink colored. It is finely tomentose and consists of small tufts of skeletal and generative hyphae that grow diagonally or perpendicularly from the pileus surface. The generative hyphae are thin-walled, hyaline, abundantly clamped, frequently branched, and 2.2-3.3 μ in diameter. These hyphae are occasionally observed among the skeletal hyphae

in dense "islands". The generative hyphae are not in definite parallel alignment but are intertangled and interwoven with each other. The skeletal hyphae are thick-walled, aseptate, light brown-colored, refractive, and 2.2-5.5 μ in diameter. Branching is very sparse and irregular, with branch lengths usually of 30-50 μ. The diameters of the branches is the same or slightly narrower than the main hyphae.

In the older parts of the basidiocarp the pileus surface becomes reddish-brown to light-brown in color and is glabrous. Zonation in which light and dark areas are formed over the surface may also occur. The skeletal hyphae predominate in the glabrous, zonate pileus surface. They are no longer in tufts but are agglutinated to form a compact, firm mass (Figure 37). The hyphae are similar in form and size to the skeletal hyphae of the marginal regions, but they differ in color. The skeletal hyphae in the older areas are very dark reddish-brown. This color is due to their dark hyphal walls, dark reddish-brown hyphal contents, and a dark-brown amorphous substance often seen among the hyphae. The intensified reddish-brown color of the hyphae results in zones that form at the pileus surface and in thecontext region (Figure 38). The zones appear to form as a result of unfavorable growth conditions such as high temperatures, desiccation, or very high light levels which may halt temporarily the radiate growth of the margin. The color of the hyphae at the pileus surface and margin

becomes intensified as a result of pigmentation of the hyphal walls and cytoplasm. A dark-colored amorphous substance may be produced by the hyphae at this time. When favorable growth conditions return, new skeletal hyphae develop behind the old hyphae and grow past them to form a new margin. If the old skeletal hyphae have not been killed, there may be renewed growth at each hyphal tip which results in a constriction in the hypha (Figure 39). The constriction forms because only the very apical portion of the hypha starts to grow. Possibly this is due to the hyphal wall being thinnest at that point.

In the oldest regions of the pileus surface, the action of weathering may bleach the color to a shade of grayish-white. The skeletal hyphae in these areas have little or no color in the upper 30-100 μ portions of their apices (Figure 40). Furthermore, the amorphous substance surrounding the skeletal hyphae has lost its brown color.

iv. The dissepiments

From the edge of the developing basidiocarp back to between 500 and 2000 μ, the margin is sterile. At the inner edge of this lower, sterile area pore development begins (Figure 41). The pore walls are composed of generative and skeletal hyphae. The development of the skeletal hyphae is similar to that in the outer edge of the margin. Thin-walled generative hyphae begin to grow downwards with skeletal hyphae developing from their terminal cells. The lower edge of the pore wall is similar to a small, concise

marginal region that shows positive geotropic response. In precisely defined areas of the lower surface, skeletal and generative hyphae grow downwards while in other areas, there is little or no downward growth of hyphae. This results in the formation of pores. The downward growth of the generative and developing skeletal hyphae is not in a definite parallel alignment: the hyphae interweave among each other as they grow in a general downward direction (Figure 42).

The skeletal hyphae in the dissepiments are more contorted and tortuous in form than the skeletal hyphae in the context. They do not show definite parallel alignment, although growth is downward in a longitudinal direction. The hyphae are thick-walled, refractive, brown-colored, and 1.7-3.9 μ in diameter which is slightly narrower than the context skeletal hyphae. The lumen is partly occluded and clamp connections are not present except where the skeletal hypha is still attached to a generative hypha. Branched skeletal hyphae are occasionally observed in the dissepiments. These branched hyphae arise from the thin-walled generative hyphae in the same manner as those in the marginal region. However, the main portion of the skeletal hypha giving rise to the branches is not as long and flexuous as the skeletal hyphae in the context. The branched, skeletal hyphae of the dissepiments appear to have a binding function in that they hold the rest of the hyphae in a coherent, tight mass. In Figure 43 the straighter and wider main portion of a skeletal hypha can be compared to

the narrower, more contorted branched portion.

The generative hyphae observed in the dissepiments were of the thin-walled and irregularly thickened types, but the irregularly thickened type was infrequently seen. The thin-walled hyphae are 1.7-2.3 μ in diameter and are frequently branched, usually near the clamp connections. The hyphal contents stain deeply with phloxine but appear hyaline when unstained. The form of the hyphae is contorted and tortuous and they are frequently seen among the skeletal hyphae. The generative hyphae are abundant along the edge of the pore walls where they give rise to the basidia that form the hymenial surface.

The basidia form as terminal cells of the generative hyphae. A palisade layer of basidia develops along the pore walls. The basidia are clavate-shaped, thin-walled, hyaline, 10-16.2 x 3.1-4.6 μ in size, and clamped at their base (Figure 44). They bear four sterigmata which are slender and 2.7-3.9 μ long. Immature basidiospores are infrequently observed on the sterigmata. The mature basidiospores are narrow-cylindric, hyaline, smooth-walled, slightly curved at one end, and measure 4.0-7.0 x 1.5-2.0 (-2.3) μ (Figure 45). The basidia line the entire pore wall except for the outer, lower edge where the skeletal hyphae protrude. Even before the pore walls have reached their maximum length, the basidia already line the walls.

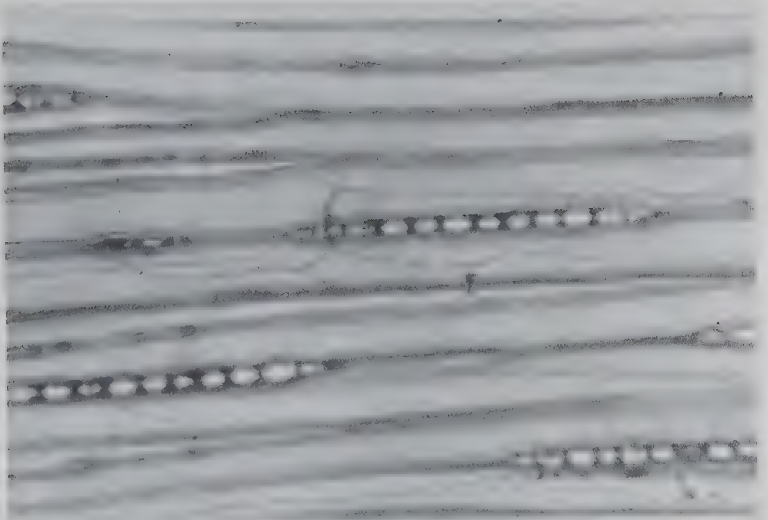
The upper parts of the older pores are often filled with thin-walled generative hyphae that are devoid of

contents (Figure 46). The presence of these generative hyphae gives the pores a "white, stuffed" appearance when viewed with the naked eye. It appears that hyphae grow into the pore space from generative hyphae lining the pore walls and directly from sterile basidia in the stuffed areas. Fertile basidia have not been observed in the stuffed portions of the pores. The function or reason for the stuffed pores is not known.

Figure 1. Branching hyphae growing through the lumina of tracheids in pine wood. Note the longitudinal direction of growth. X215.

Figure 2. Tracheids penetrated by hyphae via the bordered pits. The lowermost hypha has branched upon emergence from the bordered pit. X695.

Figure 3. Dense hyphal aggregations in the tracheids near the ray cells. X215.



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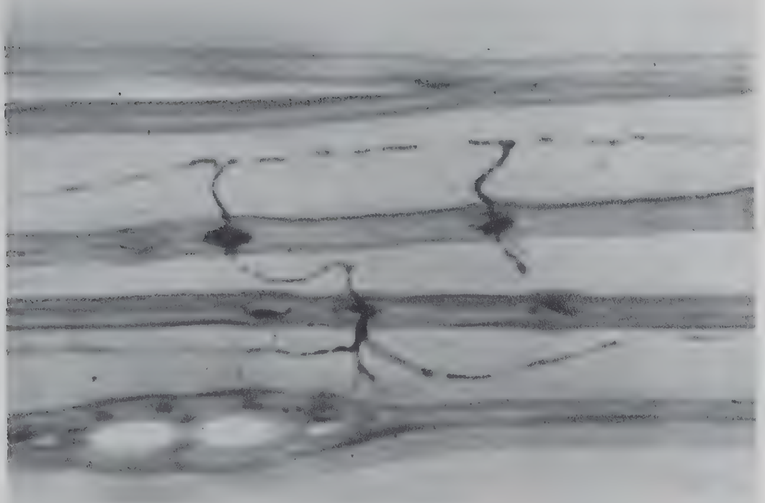

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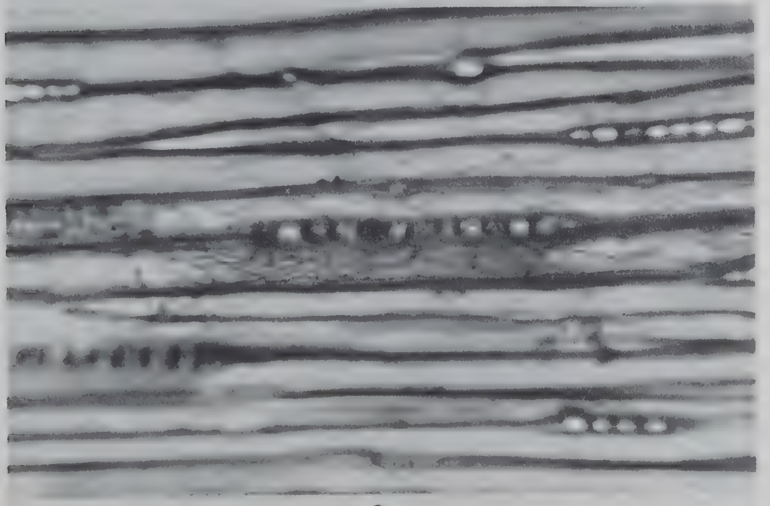

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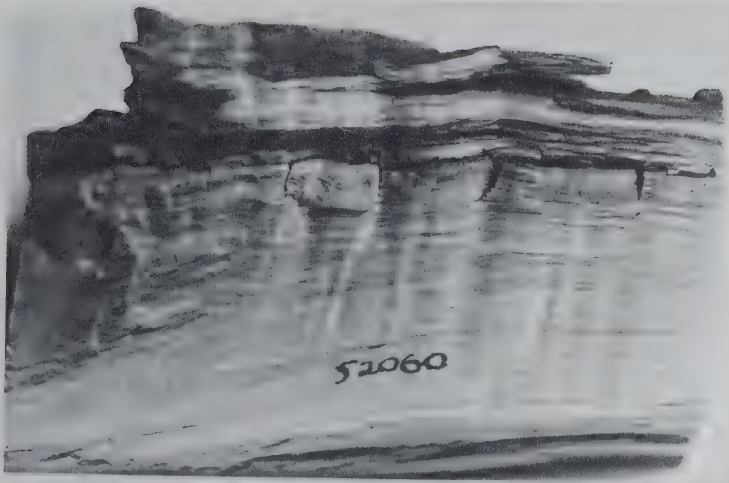
Figure 4. Brown cubical rot of *Populus* wood from Ottawa collection. DAOM 52060, X1.2.

Figure 5. Brown rot of conifer wood showing cubes that crumble easily. X7.4.

Figure 6. Tracheids near the wood surface completely filled by dense masses of thick-walled hyphae. X695.



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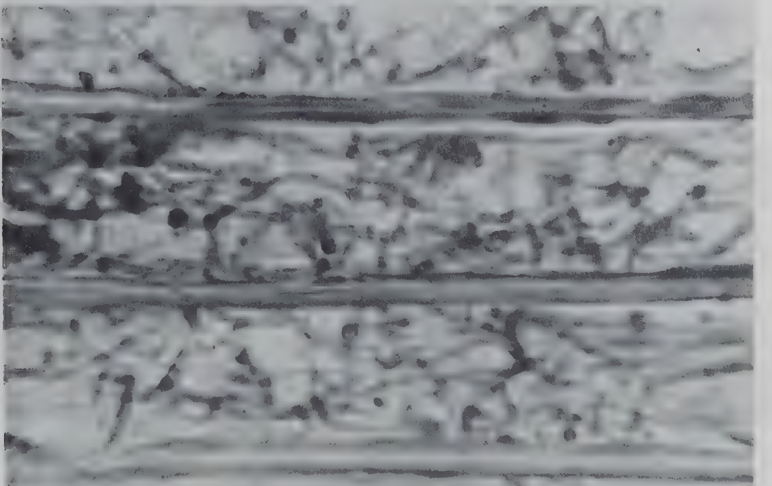

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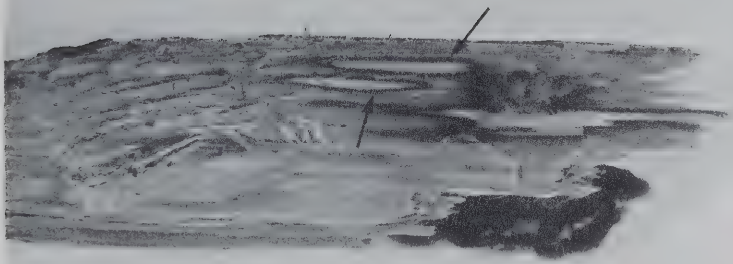
Figure 7. Thin, dark stain lines (arrows) in wood beneath *F. cajanderi* basidiocarps. X1.1.

Figure 8. Broad, dark stained area (arrow) in wood infected by *F. cajanderi*. Note the irregular edges of the stained area. X1.6.

Figure 9. Tracheids at the edge of stained area filled with dense hyphal aggregations composed of dark-brown, thick-walled hyphae. X64.5.



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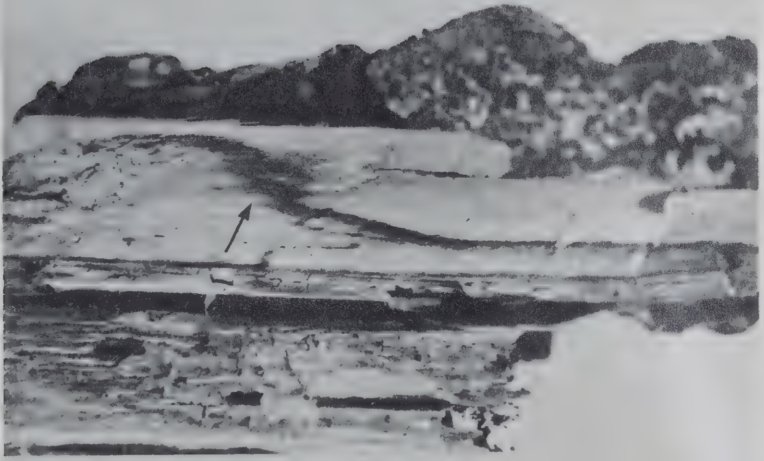

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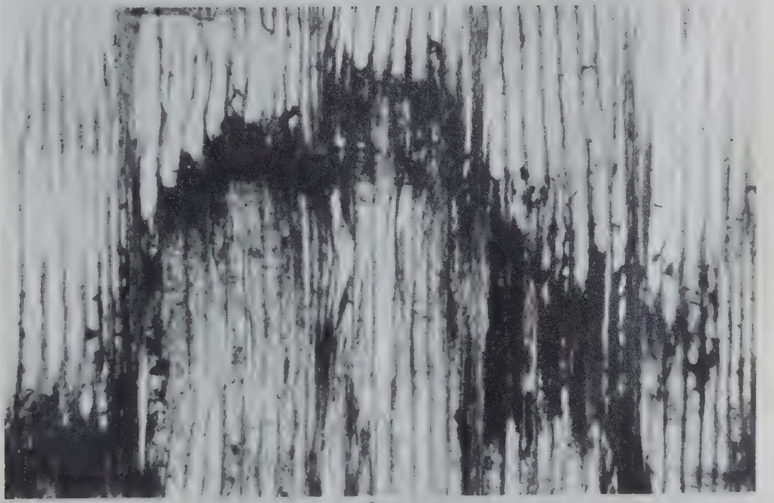

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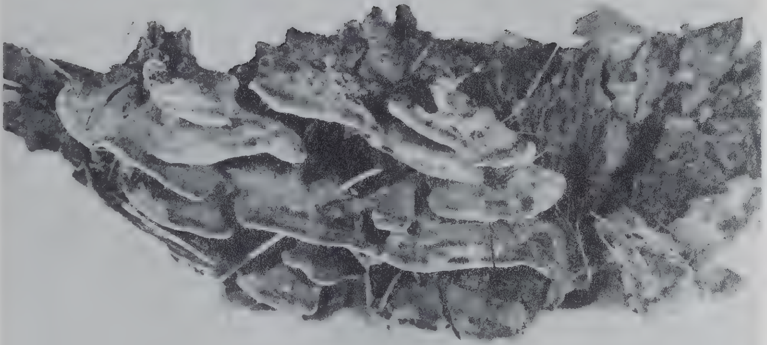
Figure 10. Laterally fused and confluent sessile basidiocarps. B-191, X0.80.

Figure 11. Fully developed, effused-reflexed basidiocarps. B-166, X0.70.

Figure 12. Circular, applanate basidiocarp from Ottawa collection. DAOM 125514, X0.65.



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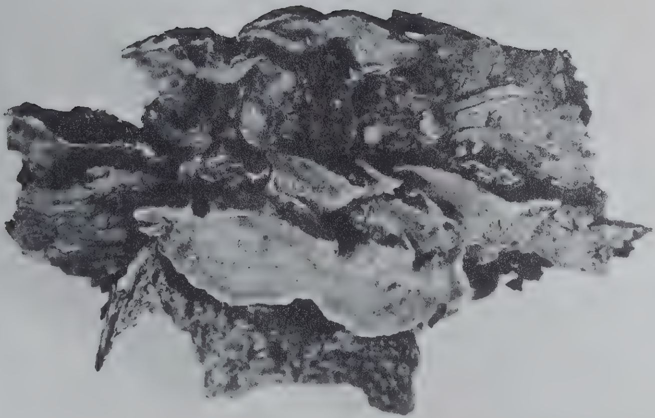

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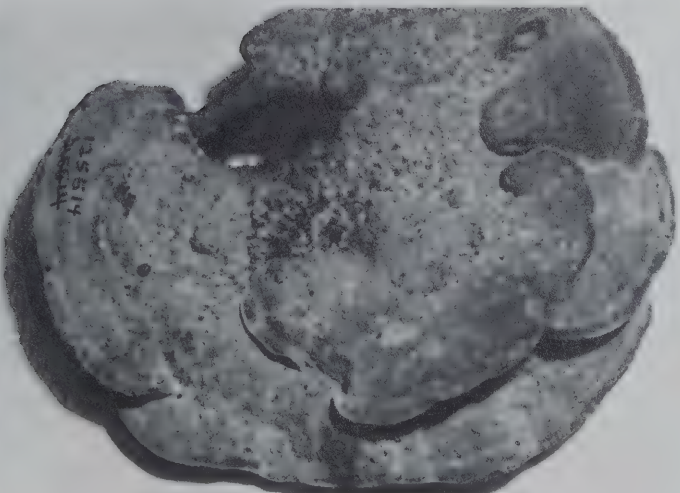

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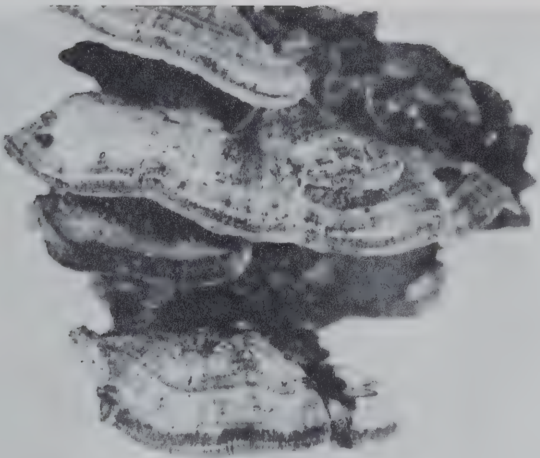
Figure 13. Fully developed, sessile, convex basidiocarps. Note the zonate, radiately fibrillose pileus surface. B-128, X0.80.

Figure 14. Fully developed, imbricate basidiocarps. Note the zonation of the pileus surface. B-3, X0.80.

Figure 15. Fully developed, resupinate basidiocarp that formed on the underside of a spruce log. B-333, X0.80.



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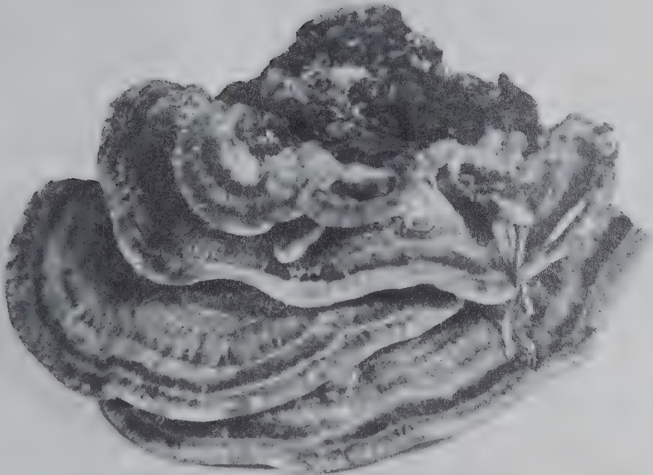

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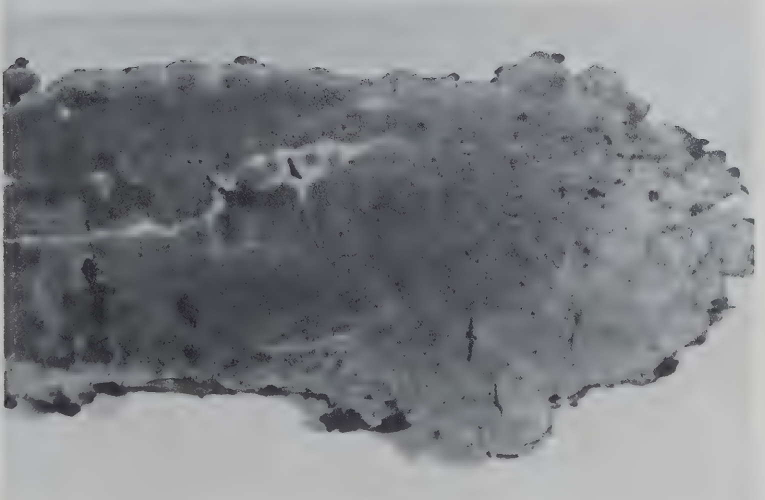

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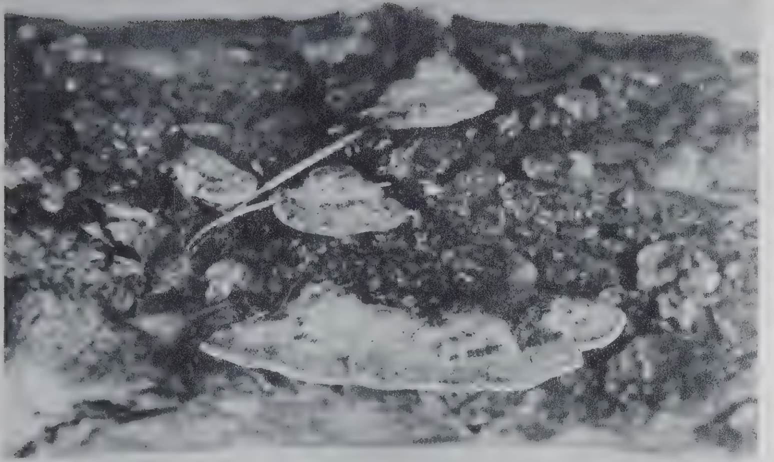
Figure 16. Sessile basidiocarps developing on the barked portion of a conifer log. B-294, X0.75.

Figure 17. Fully developed, sessile basidiocarp growing in a large crack of a debarked log. Note the lateral fusing of the basidiocarp. The cracks in the pileus formed when the specimen was dried for herbarium use. B-194, X0.55.

Figure 18. Sessile, convex basidiocarp. The zones on the pileus surface are formed by shallow furrows. DAOM 100599, X1.2.



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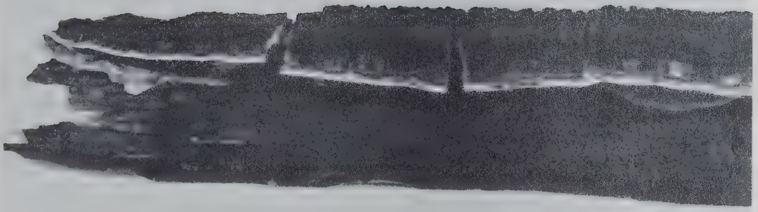

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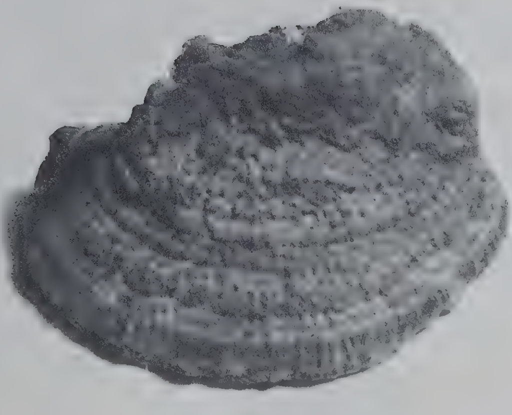

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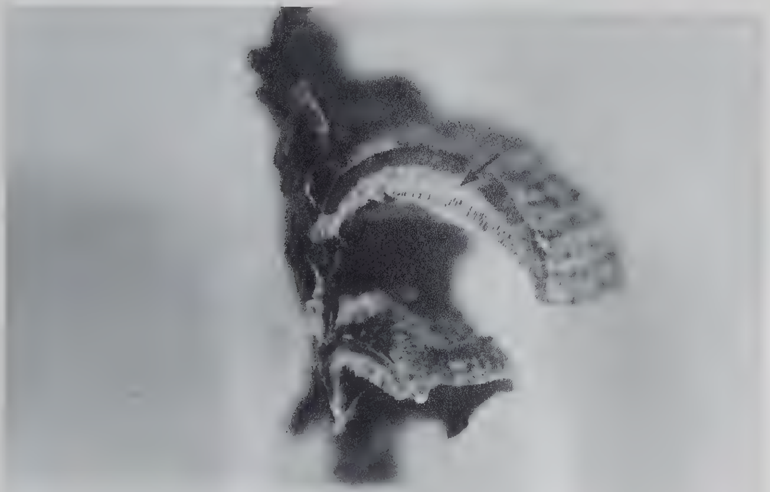
Figure 19. Cross-section of a sessile, convex basidiocarp showing the conchate form. Note the "white, stuffed" appearance of the older pores (arrow). B-128, X0.55.

Figure 20. Pore surface of a fully developed basidiocarp. Note the sub-angular pore mouths and the sterile margin to the right. X7.4.

Figure 21. Tomentose marginal region of a developing basidiocarp. X7.4.



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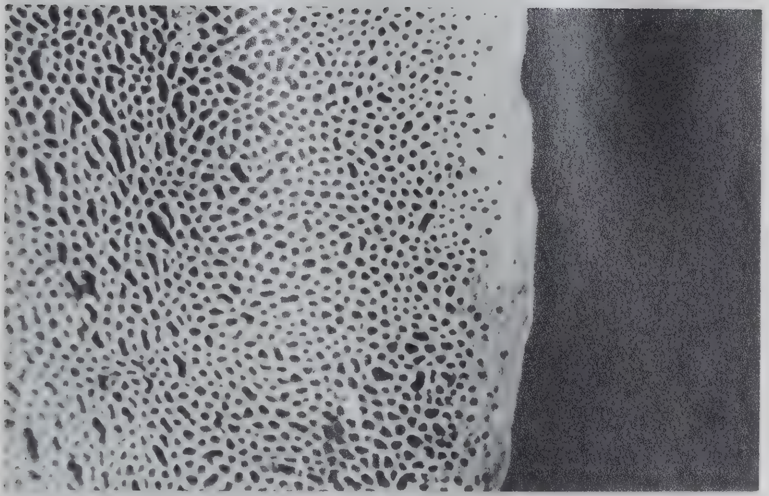

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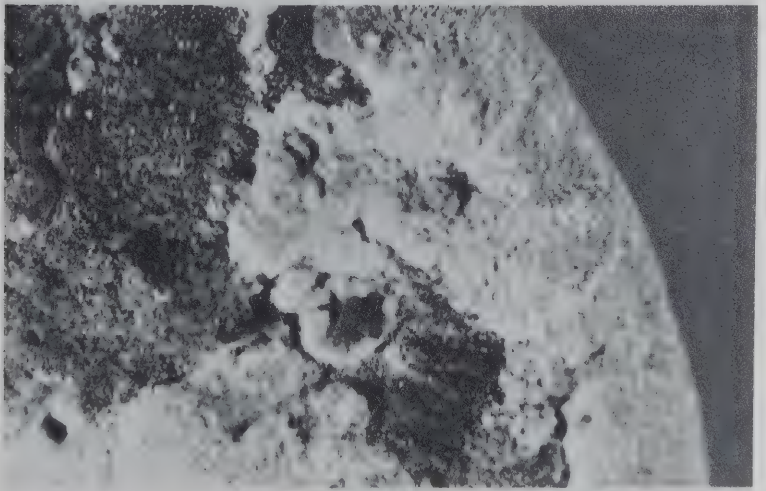

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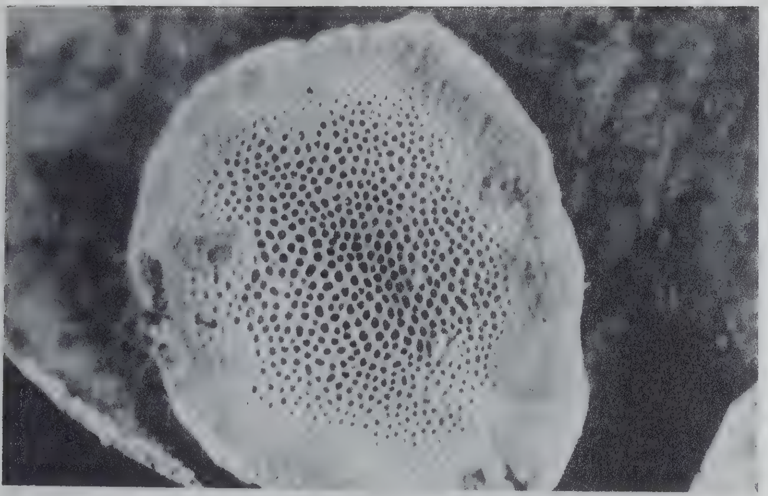
Figure 22. Developing basidiocarp showing temporary resupinate form. Note the round, thick-walled pores and the wide sterile margin. X7.4.

Figure 23. Developing effused-reflexed basidiocarp. Note the daedaloid pores of the effused portion. X7.4.

Figure 24. Cross-section of a developing basidiocarp with round margin edge and shallow pores. Note the zonation in the context. X7.4.



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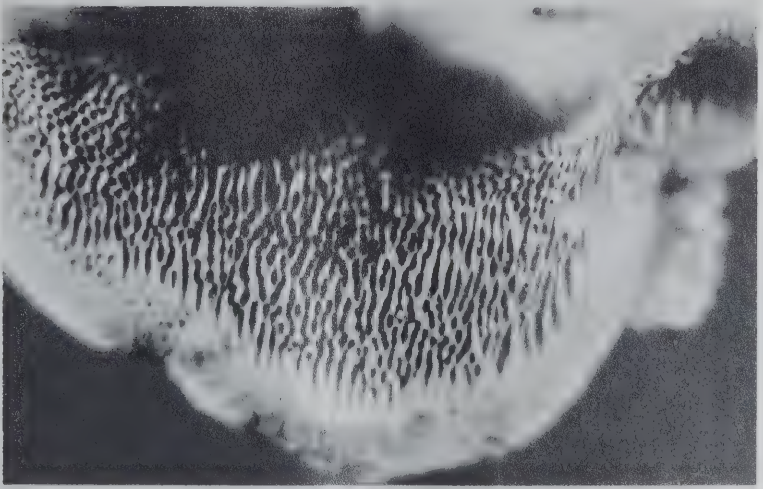

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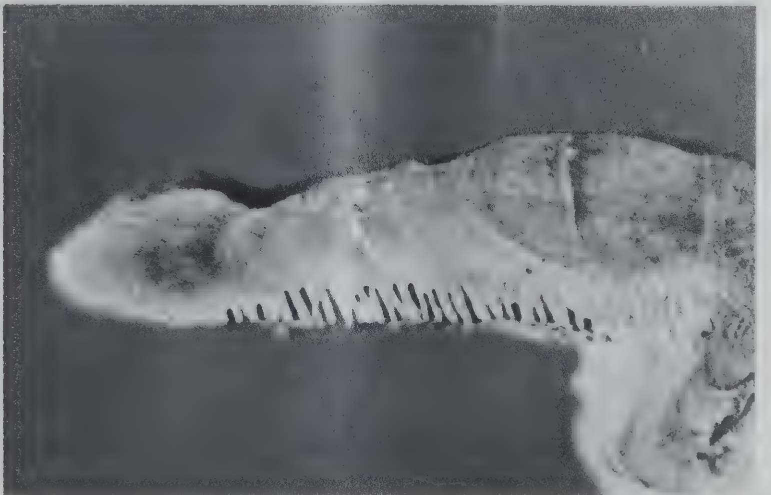

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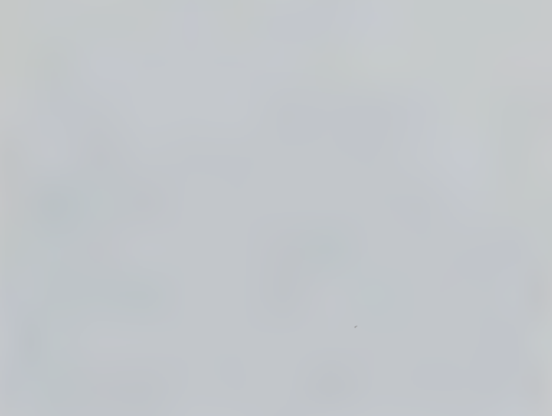


Figure 25. Mycelium near the wood surface emerging through a crack in the bark. X49.

Figure 26. Small, hemispherical primordium emerging through a crack in the bark. Note the light colored, compact surface of the primordium. X5.6.

Figure 27. Section through a primordium showing the outward growth in a radial manner. X49.

Figure 28. Primordium with a finely tomentose, rugulose surface. The upper surface is dark reddish-brown while the margin and lower surface are light-pink. X5.6.

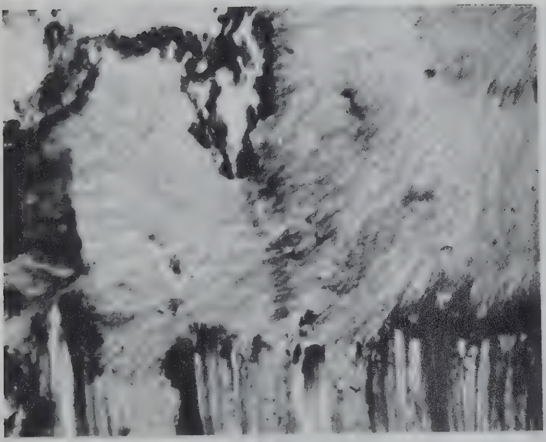
Figure 29. Vertical section through a basidiocarp of *F. cajanderi* showing the four regions examined. Note the renewed growth in the marginal region. X5.6.

M - margin.

C - context.

P - pileus surface.

D - dissepiments.



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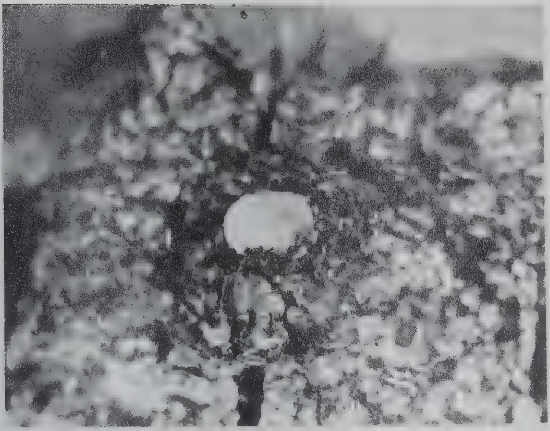

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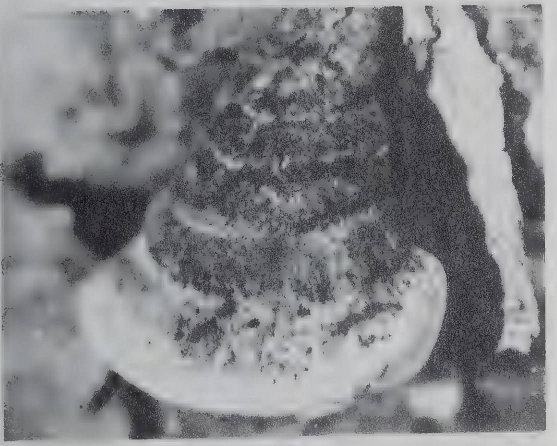

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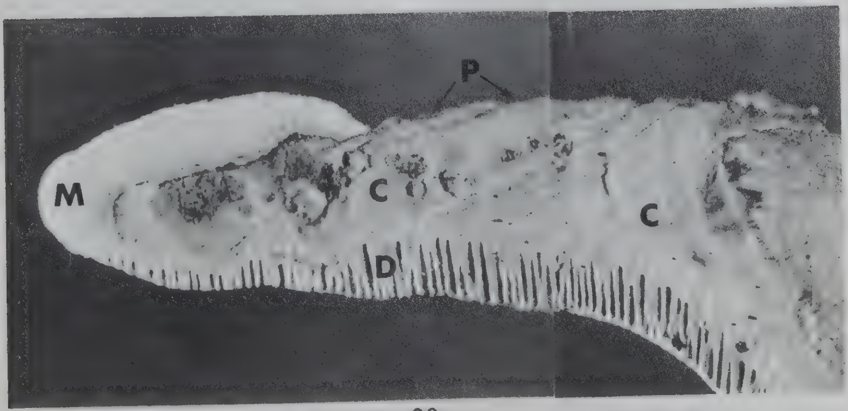

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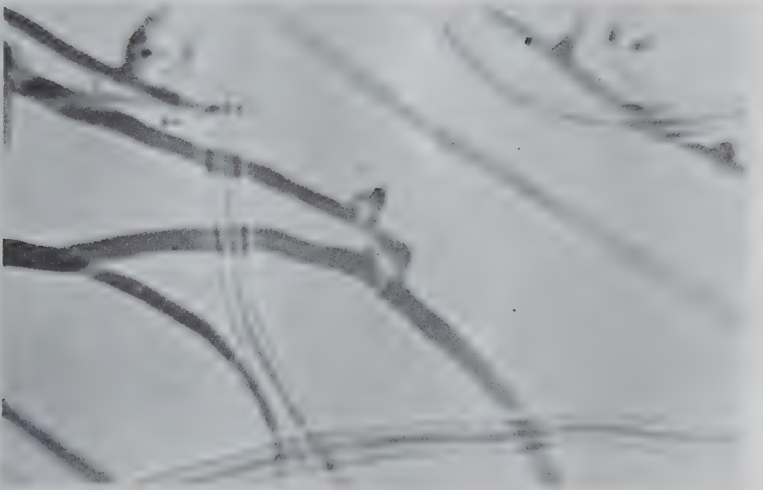
Figure 30. Generative hypha from the marginal region showing a branch arising directly from clamp connection. X1450.

Figure 31. Developing skeletal hypha (arrow) showing the proximal end attached to generative hypha by clamp connection. X1450.

Figure 32. Parallel arrangement of developing skeletal hyphae in the marginal region. X64.5.



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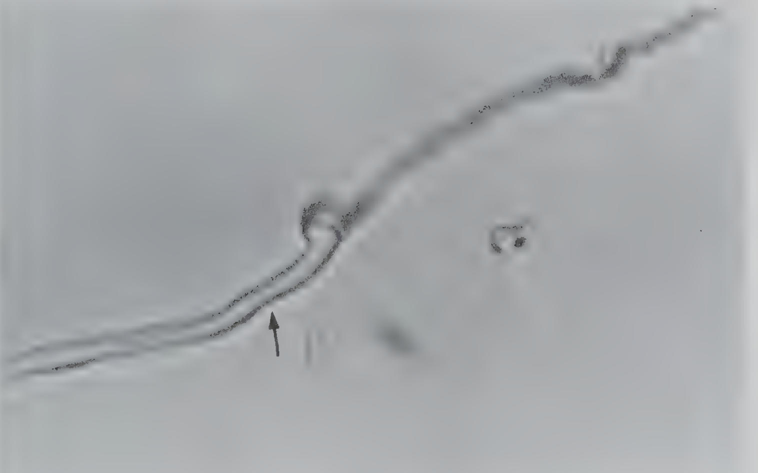

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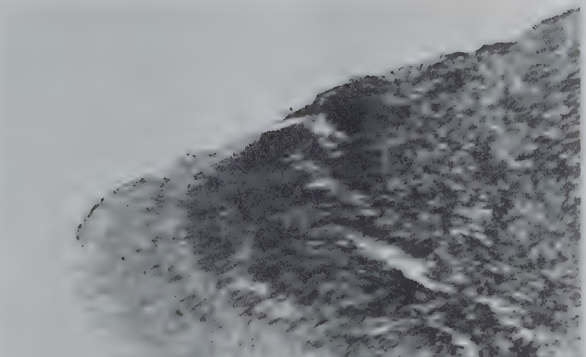

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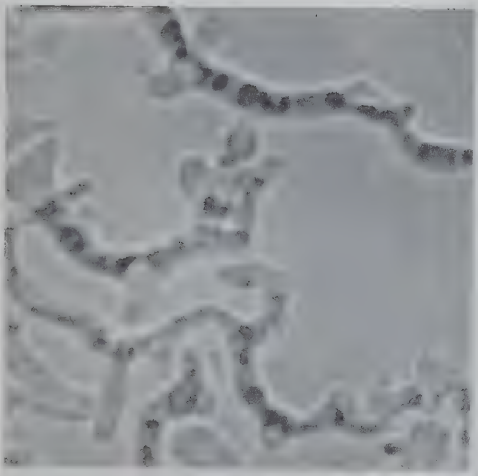
Figure 33. Generative hyphae showing irregularly thickened walls and contorted, tortuous growth form. X1450.

Figure 34. Broken generative hypha with hooked portion of clamp connection (arrow). X1450.

Figure 35. Branched end of a skeletal hypha from the context region. X1450.

Figure 36. Long, flexuous skeletal hyphae in parallel alignment in the context region. X347.



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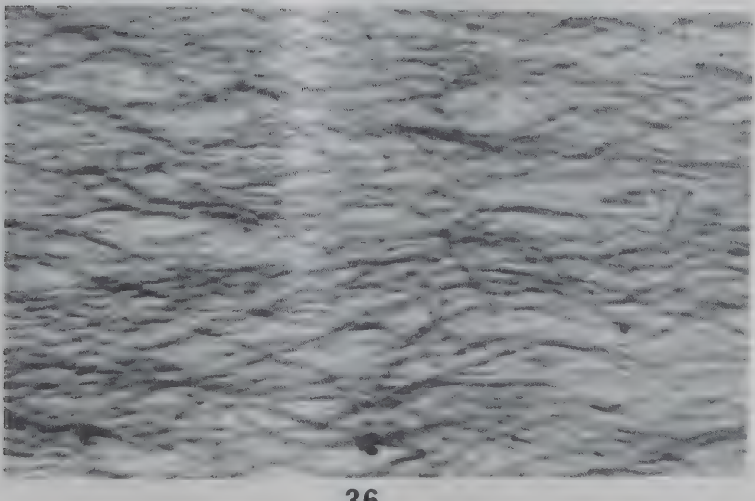

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Figure 37. Compact, firm mass of agglutinated skeletal hyphae at the pileus surface. X180.

Figure 38. Cross-section of pileus surface and context showing zones. Hyphae in the dark areas are dark reddish-brown. X64.5.

Figure 39. Skeletal hypha from pileus surface showing a constriction where growth was halted temporarily. X1480.

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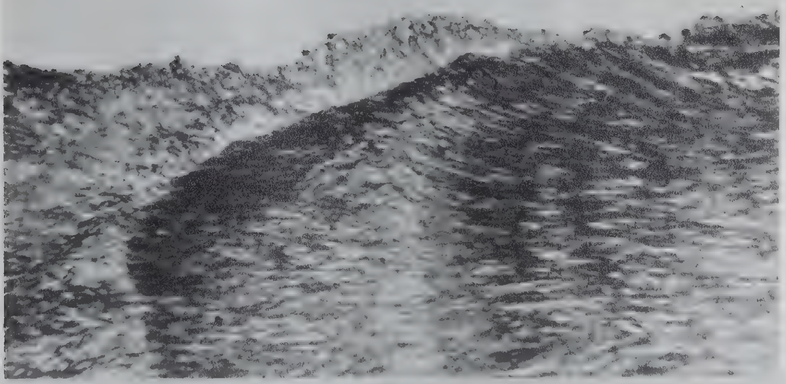

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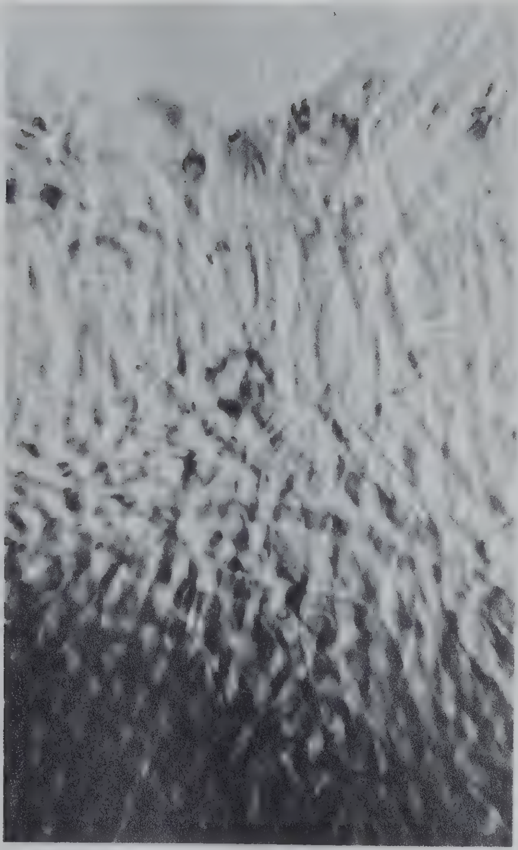
Figure 40. Skeletal hyphae at the pileus surface. Note the colorless, refractive hyphal walls and the dark pigmented walls approximately 50 μ below. X580.

Figure 41. Beginning of pore development at the inner edge of the lower sterile margin. X215.



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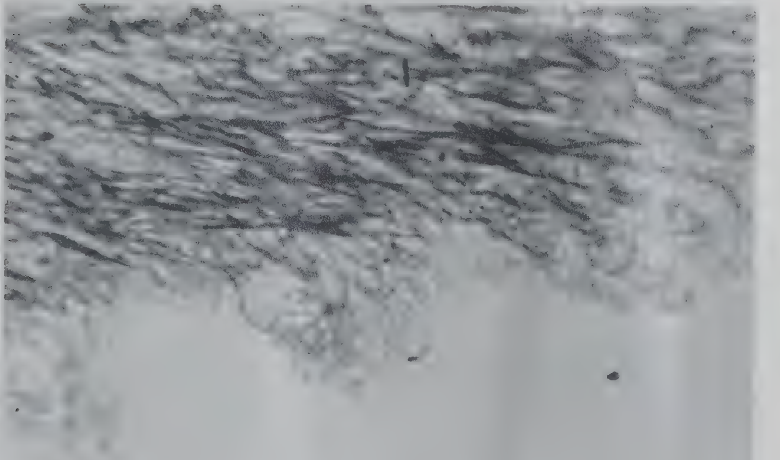

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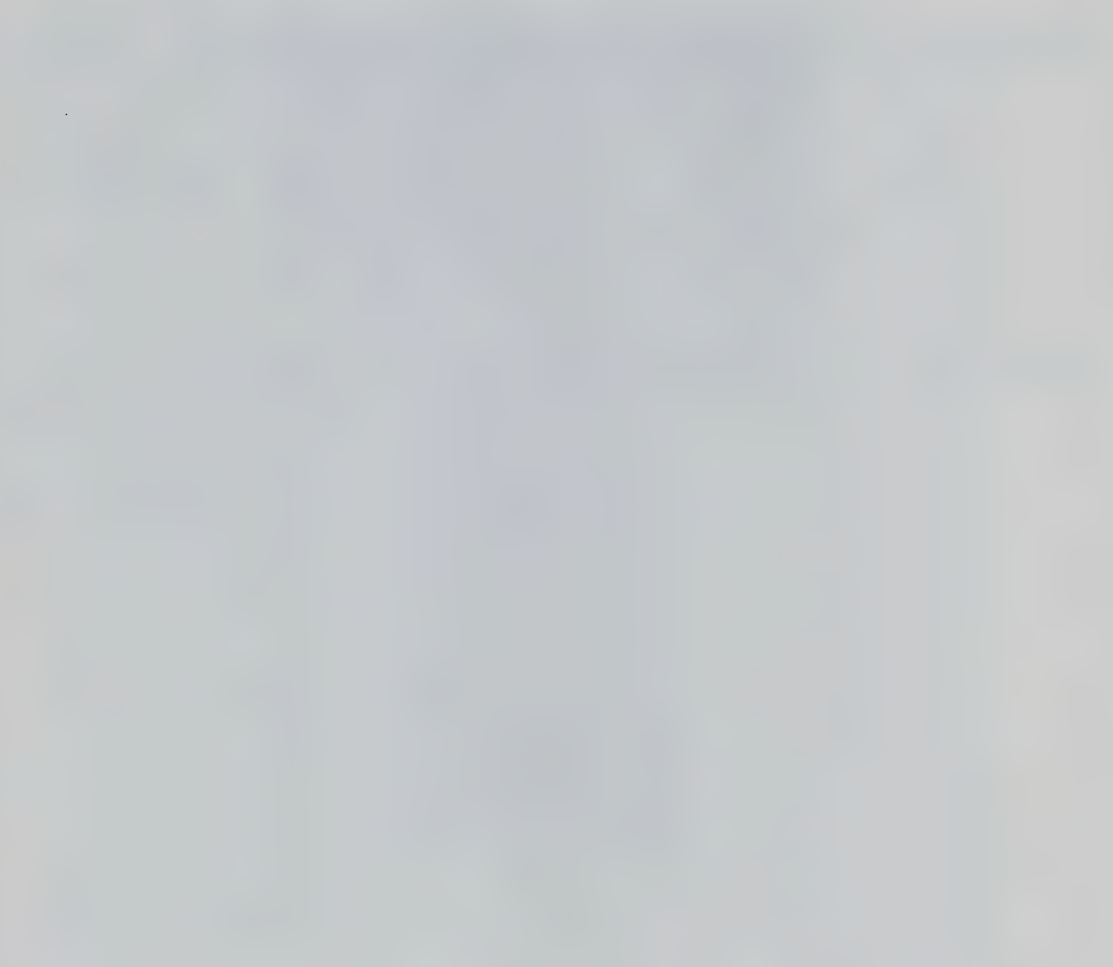

Figure 42. Skeletal hyphae of the dissepiments. Note the interweaving and the slightly narrower diameter of the hyphae compared to the context skeletal hyphae. X215.

Figure 43. Skeletal hypha from the dissepiments. The straighter, wider hypha forms the main portion of the narrower, more contorted branch. X3200.

42

43

Figure 44. Clavate, thin-walled, immature basidia. Each basidium bears a clamp connection at its base. X1480.

Figure 45. *F. cajanderi* basidiospores stained with phloxine. Note the narrow-cylindric shape, slight curve at one end, and smooth walls. X1480.

Figure 46. Older pores of basidiocarp stuffed with thin-walled generative hyphae. X64.5.

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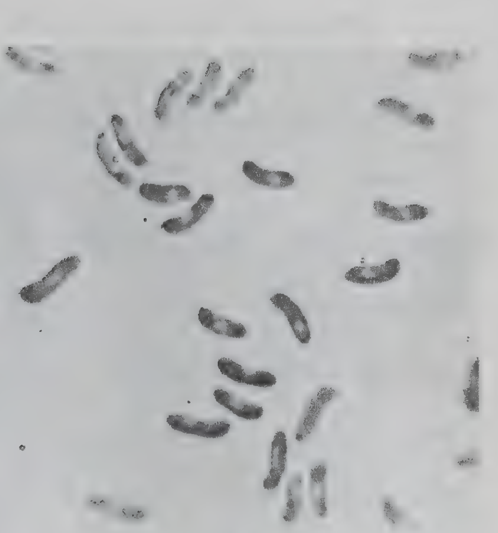

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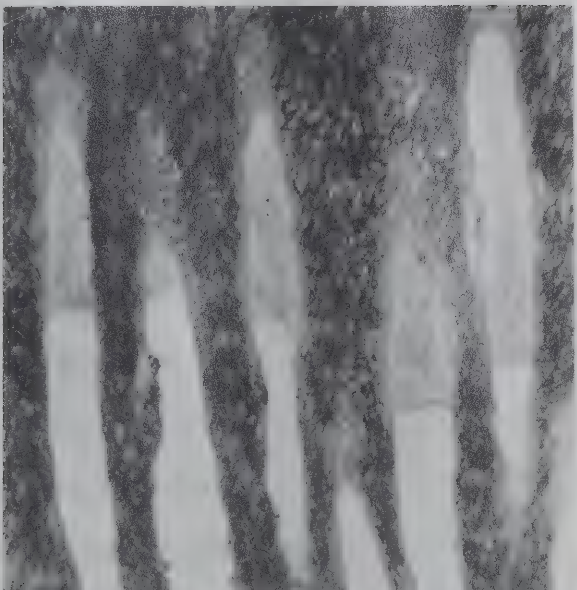

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B. Development of mycelium and basidiocarps in culture

In the descriptions of the mycelium and basidiocarps formed in culture, the terms of Nobles (1958, 1965) were used to describe the macroscopic and microscopic characters. Nodose-septate hyphae are hyphae bearing clamp connections. The hyphal walls may be thin or thick. Fiber hyphae are "hyphae with thick refractive walls, hyaline or brown, and lumina narrow or apparently lacking." These hyphae "arise usually as the elongated terminal cell of a hypha and are thus aseptate." Branching may be absent, rare, or frequent. The term refractive is used here to describe hyphae whose walls are shiny-appearing and unstained with phloxine.

1. Growth of the vegetative mycelium

For each of the three types of cultures the growth and structure of the vegetative mycelium will be described in two sections: macroscopic characters and microscopic characters. The vegetative or assimilative mycelium is considered here to be the growing or nutrient-absorbing mycelium. Hyphal swellings on the thin-walled hyphae called chlamydospores in this thesis will be described with the vegetative mycelium.

a. Agar Culture - Macroscopic characters

The initial growth of the vegetative mycelium in agar culture is cottony to cottony-floccose, moderately dense, and white. The colony edge is uneven and consists of fine, branching hyphae that grow in a radiate manner. These

hyphae are flat on the agar and are difficult to see unless observed against a bright light. The cottony to cottony-floccose aerial mycelium forms within 2-3 mm of the colony edge. The general appearance of the mycelium remains the same until the colony covers the Petri plate. The growth rate of *F. cajanderi* is moderate. At room temperature 8.5 cm plastic Petri plates are covered within 18 days while 9 cm glass Petri plates are covered within 20 days. Once the plate has been nearly covered a change in the appearance of the mycelium occurs. At the plate edges the mycelium becomes dense, thick, compact, and felty. The amount of change that occurs is variable and may range from a slight increase in mycelial density (Figure 49) to a large increase in mycelial density (Figure 50). The felty mycelium becomes light-pink to pinkish-brown colored while the cottony to cottony-floccose mycelium in the central portions remains white. The width of the compact, felty areas ranges from less than 0.5 cm to 3 cm. The mycelium at the plate edges may remain cottony to cottony-floccose and white with only scattered portions of the mycelium becoming dense and felty. In some instances the mycelium may form felty, compact areas on the Petri plate walls. The felty areas of mycelium are considered to be primordia since pore development occurs there. The structure of these areas will be discussed in the section on fruiting development in agar culture. The agar below the cultures does not become discolored and there is no oxidase reaction.

The odor of the cultures is fragrant and is like sweet apples.

Microscopic characters

The mycelium at the edge of the agar cultures is composed of thin-walled, nodose-septate hyphae that are frequently branched and of variable diameter ranging from 1.4-4.2 µ. Branches occur along all parts of the hyphae and are the same diameter or slightly narrower than the main hyphae. The contents of the hyphae are granular appearing, homogeneous, and hyaline when unstained. The hyphae are growing on the agar surface and submerged slightly in the agar. Within 2-3 mm of the front edge of the cultures aerial mycelium has developed. This mycelium is composed of nodose-septate hyphae and fiber hyphae. The nodose-septate hyphae are thin-walled, granular appearing, abundantly clamped, and frequently branched. They range in diameter from 1.1-2.8 (-3.3) µ. These hyphae are not organized in any arrangement but are intertangled with each other and the fiber hyphae. The fiber hyphae near the edge of the mycelium are occasionally seen still attached to the thin-walled, nodose-septate hyphae from which they arose. A fiber hypha develops from a thin-walled, nodose-septate hypha as a long, flexuous, hyaline, granular appearing, aseptate terminal cell. The diameter of the developing fiber hypha is initially the same as that of the nodose-septate hypha from which it is arising. As the developing fiber hypha grows, its diameter gradually increases until

it reaches a diameter 2-3 times that of the nodose-septate hypha. In a developing fiber hypha measuring over 590 µ in length the proximal end was 1.5 µ in diameter while the central and apical portions were 2.5 µ in diameter. Wall thickening occurs in the developing fiber hypha when it is 50-100 µ in length. The central portions of the fiber hypha thicken first and this process continues towards each end of the hypha. As wall thickening occurs the lumen becomes narrower and the contents appear as faintly stained areas in the hypha (Figure 51). The thickened walls appear refractive, colorless, and may reach 1.0 µ in thickness. When the fiber hypha is fully developed it may measure over 1500 µ in length. The apical and proximal ends of the fiber hypha are not as thick-walled as the central portions although the lumen is narrow and lightly stained. In the central portions of the fiber hypha the lumen may be completely occluded or reduced to a very thin, granular appearing, faintly stained line running down the center of the hypha. Branching occasionally occurs at the apical end of the fiber hypha (Figure 52) or in the central portions. Two to four branches may arise from a fiber hypha. The branches are the same diameter or slightly narrower than the main hypha from which they arose. The branches may reach lengths of over 150 µ. The hyphal walls and lumen contents of the branches are similar to the main fiber hypha. In the aerial mycelium the fiber hyphae are the predominant hyphal type observed. These hyphae are

long, flexuous, and loosely intertangled and interwoven with each other and the nodose-septate hyphae (Figure 53). The intertangled and interwoven fiber hyphae and nodose-septate hyphae are responsible for the cottony to cottony-floccose texture of the central areas of the aerial mycelium.

The nodose-septate hyphae in the central portions of the aerial mycelium are thin-walled or irregularly thickened. The thin-walled, nodose-septate hyphae are similar in appearance to the nodose-septate hyphae observed near the edge of the mycelium. The hyphae are granular appearing, 1.1-3.3 μ in diameter, abundantly clamped, frequently branched, and hyaline when unstained. They do not show any organization but are loosely intertangled and interwoven among the fiber hyphae and the irregularly thickened, nodose-septate hyphae. In the aerial mycelium the irregularly thickened, nodose-septate hyphae (Figures 54, 55) are frequently observed. These hyphae develop from the thin-walled, nodose-septate hyphae. The first indications of wall thickening are the clamp connections becoming swollen, solid, and refractive and the formation of short, solid, refractive protuberances along the hyphal walls. The walls later become irregularly thickened so that the lumen appears narrow and sinuous. The lumen contents are granular appearing and stain deeply with phloxine while the thickened walls are colorless. The irregularly thickened, nodose-septate hyphae are 1.7-4.2 μ in diameter, frequently branched, and abundantly clamped.

The hyphae often become separated at the clamp connections where attachment appears to be weak. Hyphal ends are frequently seen bearing the hook portion of a broken clamp connection (Figure 54). The swollen, thick-walled clamp connections suggest the appearance of a ball-and-socket structure as Nobles (1965) has observed. The irregularly thickened, nodose-septate hyphae in the aerial mycelium are in scattered "islands" intertangled among the thin-walled, nodose-septate hyphae and the fiber hyphae.

The submerged mycelium is composed of thin-walled, nodose-septate hyphae that are profusely branched, abundantly clamped, 1.4-4.2 µ in diameter, granular appearing, and deeply stained with phloxine (Figure 56). The hyphae are hyaline when unstained. In older cultures irregularly thickened, nodose-septate hyphae are frequently observed in the submerged mycelium. These hyphae are identical to the irregularly thickened hyphae of the aerial mycelium. Chlamydospores are occasionally observed among the submerged mycelium. They occur frequently in some isolates and rarely or not at all in other isolates. The chlamydospores are elongate to ellipsoidal in shape, 13.2-27 x 5.6-7.5 µ in size, thin-walled, granular appearing, and deeply stained with phloxine (Figure 57). They are hyaline when unstained. The chlamydospores form as intercalary cells in the thin-walled, nodose-septate hyphae.

b. Still culture - Macroscopic characters

In all still cultures the inoculum plug sank soon

after inoculation and remained submerged throughout the incubation period. The first 7-10 days of growth produces only submerged mycelium which forms a spherical, dense, yellowish mass around the inoculum plug. The submerged mycelium is fragile and teases apart readily. The malt extract medium is clear and yellow which gives the mycelium a yellowish tinge. The submerged mycelium appears white when observed in distilled water. After 14 days of growth the submerged mycelium has increased in size, measuring up to 4 cm across. The mycelium is in irregular, somewhat spherical masses with ragged and uneven edges. In the great majority of flasks two or more masses of mycelium had developed. They had started from fragments of mycelium that had separated from the main inoculum mass.

Floating mats of aerial mycelium have developed by this time and are formed when the submerged mycelium meets the liquid-air interface of the medium. The margins of the floating mats are very irregular and sparse. The central portions are denser, powdery to floccose in texture and white in color (Figure 58). The liquid medium remains clear and yellowish with no evidence of discoloration. The odor of the cultures is fragrant and is like sweet apples. After 25 days of growth the aerial mycelium covers 1/3 to 1/2 of the medium surface (Figure 59). The margins of the mats remain sparse, white, and thin while the central portions are thicker, denser, and cottony to cottony-floccose in texture. Most of the mycelium is white except where small,

hemispherical mounds of mycelium have formed. The mycelial mounds are initially white and later become light-pink to brown-colored. These mounds are considered to be primordia and their formation and structure will be discussed in the section on fruiting development in still culture. The aerial mycelium grows both on the liquid surface and up the inner walls of the flasks. The mycelium growing over the walls of the flasks is similar in appearance to the aerial mycelium covering the medium. The submerged mycelium and the liquid medium is unchanged from before. The culture odor of sweet apples also remains unchanged.

Microscopic characters

In all stages of growth, the submerged mycelium is similar in appearance. The only hyphal type observed is the nodose-septate hyphae. These hyphae are thin-walled, but in older cultures the walls become irregularly thickened. The contents of the thin-walled hyphae are granular, homogeneous, and hyaline. Branching is profuse and occurs along all parts of the hyphae. In some instances a branch may arise directly from a clamp connection (Figure 60). The branches are the same diameter or slightly narrower than the main hyphae which range in diameter from 0.9-2.6 (-4.4) µ. The hyphae are loosely intertangled and interwoven with each other and are not in any definite organization.

Thin-walled chlamydospores are occasionally observed developing as intercalary cells of the thin-walled, nodose-

septate hyphae. Their appearance is irregular; some isolates form many chlamydospores while other isolates form few or none at all. The chlamydospores are ellipsoidal to elongate in shape (Figures 61, 62). Occasionally the chlamydospores form as part of the fork of branching hyphae. These chlamydospores are somewhat triangular in form with three branches arising from the walls. The chlamydospore contents are granular appearing, homogeneous, and hyaline. The contents stain deeply with phloxine. The sizes of the chlamydospores range from 8.8-22.9 x 4.4-12.3 µ. The chlamydospores form in all areas of the submerged mycelium and are often observed within ten days of growth. They begin development by the formation of a simple septum in a hypha. The hypha then becomes swollen and a second simple septum forms at the other side of the developing chlamydospore. The hypha continues to swell until the fully developed chlamydospore has formed. Additional simple septa may also be laid down on either side of the chlamydospore. It is not unusual to observe 3 or 4 simple septa on each side of a chlamydospore (Figure 62).

The irregularly thickened, nodose-septate hyphae develop in the older cultures. These hyphae form from the thin-walled, nodose-septate hyphae in all parts of the submerged mycelium. The first stage of wall thickening is the clamp connections becoming swollen, refractive, and solid and the formation of small, refractive, unstained protuberances along the hyphal walls. The lumen passing

through a swollen clamp connection may become completely occluded or remain as a thin, granular appearing, hyaline line. The irregularly thickened hyphae often break or separate at the clamp connections where attachment appears to be weak. As wall thickening continues along the hyphae the lumina becomes thinner and more sinuous in appearance. The thickened walls are unstained and refractive. They vary in thickness, some becoming 1.3 μ or more thick. The diameter of the irregularly thickened hyphae ranges from 0.9-4.4 μ which is the same as the thin-walled hyphae. The general form of the irregularly thickened hyphae is similar to the thin-walled, nodose-septate hyphae.

The aerial mycelium is composed of two types of hyphae: the nodose-septate hyphae (thin-walled and irregularly thickened) and the fiber hyphae.

The nodose-septate hyphae in the aerial mycelium are predominantly irregularly thickened and are identical in appearance to the irregularly thickened hyphae in the submerged mycelium. The hyphal contents stain deeply with phloxine and appear granular and homogeneous. The walls are unstained, refractive, and solid appearing. The lumen is narrow and sinuous, and occasionally is completely occluded. Clamp connections are frequent and appear swollen, refractive, and solid. The hyphae are often broken or separated at the clamp connections leaving the hyphae with the hooked portions at one end. The hyphal walls also have small, refractive, solid protuberances

that give the hyphae a "bumpy" appearance. Branching is frequent and usually occurs near clamp connections. The branches are slightly narrower or the same diameter as the main hypha from which they arose. Hyphal diameters range from 0.9-4.4 μ. No organization or pattern of arrangement of the irregularly thickened hyphae is observed. They are found in scattered "islands" among the fiber hyphae in densely intertangled and interwoven masses. The thin-walled, nodose-septate hyphae are similar to those observed in the submerged mycelium. These hyphae are frequently branched and bear numerous clamp connections. The hyphal contents are granular appearing, homogeneous, and hyaline when unstained. The hyphal diameters are narrower than the submerged hyphae. The hyphae in the aerial mycelium range from 0.9-2.6 μ in diameter.

The fiber hyphae are the predominant hyphal type observed forming well over half of the aerial mycelium. These hyphae are long, flexuous, 1.1-3.6 μ in diameter, aseptate, and densely intertangled with each other. The intertangling of the fiber hyphae produces the characteristic coherent, cottony-floccose texture of the aerial mycelium. The hyphal walls are thick, up to 1 μ, refractive, and brown-colored in KOH. The lumen is usually seen as a thin, granular line running down the center of the hypha. Occasionally the lumen is completely occluded. Branching is infrequent but when branching does occur, there are usually a number of branches (2-4) formed along a

relatively short length of hypha (Figure 63). The fiber hyphae develop from the thin-walled, nodose-septate hyphae in the same manner as those in agar cultures. They begin to form soon after the nodose-septate hyphae of the submerged mycelium reach the liquid-air interface of the medium. The terminal cell of a thin-walled, nodose-septate hypha gives rise to a long, flexuous, aseptate hypha that initially undergoes wall thickening in the central portions. The proximal end of the developing fiber hypha is attached to the nodose-septate hypha by a clamp connection. The hooked portion of the clamp connection is on the nodose-septate hypha. The apical end and the proximal end of the developing fiber hypha are deeply stained and thin-walled (Figure 64). As the hypha develops, the walls gradually thicken progressively outwards from the central portions to both ends. The diameter of the fully developed fiber hypha is variable along its length. When the fiber hypha begins development it will be the same diameter as the thin-walled, nodose-septate hypha from which it is forming. As growth continues the diameter increases until the apical and central portions may be 2-3 times the diameter of the proximal end. The apical end of the fully developed fiber hypha is thin-walled and the lumen is seen as a granular, faintly stained area in phloxine. The proximal end of the hypha is thick-walled with a narrow lumen. The nodose-septate hypha giving rise to the fiber hypha often becomes irregularly thickened and separated from the fiber hypha

at the clamp connection. In rare instances, the nodose-septate hypha is observed still attached to the fully developed fiber hypha. At this stage the nodose-septate hypha is either irregularly thickened or the walls remain thin but the hypha is devoid of contents.

c. Wood-block culture - Macroscopic characters

The descriptions of the wood-block cultures will be confined to the mycelium growing on the wood blocks. The morphology of the mycelium on the agar was similar to the agar cultures that have already been described. The vegetative mycelium completely covered the blocks except where primordia and basidiocarps had formed. The construction and formation of these structures will be described in the section on fruiting development in wood-block culture.

The appearance of the vegetative mycelium on the wood blocks was similar in cultures grown at room temperature and at 20°C. There was also no difference observed between mycelium growing on the spruce, pine, and aspen poplar blocks. The mycelium was white to light-brown, compact, felty to cottony-floccose, and sparse to dense (Figures 65, 66, 67). The top surfaces of the blocks were often covered with more mycelium than the sides. This was due to the presence of numerous primordia and basidiocarps on the top surfaces. All mycelial growth was attached very superficially to the wood blocks, since the mycelium was easily separated from the blocks. The wood immediately beneath the surface mycelium was heavily decayed and medium to

dark-brown colored. It was extremely soft, moist, and somewhat sticky. The wood crumbled easily under pressure. Only the outside 1-2 mm of the wood surface was soft, deeper into the blocks the wood was much firmer, although still medium to dark-brown colored.

The contamination-control blocks were light-brown colored, slightly moist, and hard. No growth occurred in the spruce and pine control blocks during the 12 month incubation period. The aspen poplar control blocks became contaminated by *Penicillium* after 4 months and were discarded.

The odor of the wood-block cultures was variable and no correlation was noted between the different types of wood and a particular odor. Most cultures were slightly fragrant and somewhat fruity. Some cultures had a distinct sweet apple odor while others, notably the spruce wood cultures, had a slight "woodsy" odor.

Microscopic characters

The mycelium within the spruce and pine wood blocks was similar to the mycelium observed in the naturally-infected wood. The hyphae were thin-walled, clamped, often branched, granular appearing, and 0.9-2.6 μ in diameter. In some instances, the hyphae had "medallion" calmps which were identical to those observed in the naturally-infected wood. Dense hyphal aggregations were observed near the ray cells, and the tracheids were penetrated via the bordered pits and by bore holes. The hyphae in these aggregations

were thin-walled, clamped, and granular appearing. Thick-walled, brown-colored hyphae were not observed filling the tracheids as was noted in the naturally-infected wood. The firm wood deeper in the wood blocks tore easily when sectioned in a similar manner to naturally-infected wood that was heavily decayed. The outer 1-2 mm of the blocks could not be sectioned because of its very soft and crumbly nature. In the aspen poplar wood the hyphae grew in a sinuous manner throughout the vessels. Branching occurred frequently and often took place where dense hyphal aggregations had formed. The appearance of the hyphae was similar to those found in coniferous wood.

There was considerable variation in the amounts of different hyphal types observed in the vegetative mycelium growing over the wood surface. Both nodose-septate hyphae and fiber hyphae were seen but the relative amounts varied between individual cultures. The nodose-septate hyphae were usually of the thin-walled type and were similar to those seen in agar culture and still culture. They were granular appearing, hyaline when unstained, 1.1-3.3 (-4.4) μ in diameter and frequently branched. The branches were the same diameter or slightly narrower than the main hyphae. The thin-walled hyphae were densely intertangled and interwoven with each other and the fiber hyphae. The clamp connections of the thin-walled, nodose-septate hyphae were usually of the normal type but occasionally the "medallion" type was observed (Figure 68). Thin-walled chamydospores

were infrequently observed developing as intercalary cells of the nodose-septate hyphae. Their appearance was irregular and the number formed varied from culture to culture. Chlamydospores were produced by all three isolates in the three types of wood-block cultures. However, no isolate or wood-block culture consistently produced numerous chlamydospores. The chlamydospores were similar to those formed in agar culture and still culture. They were elongate to ellipsoidal in shape, granular appearing, hyaline when unstained, and 7.7-16.5 x 4.4-7.0 μ in size.

Irregularly thickened, nodose-septate hyphae were occasionally observed in the vegetative mycelium. They were similar to those found in agar culture and still culture. The hyphae were 2.2-4.4 μ in diameter and had refractive, colorless walls and granular appearing, narrow, sinuous lumina. The refractive, solid, swollen clamp connections were of the normal type and short, refractive, solid protuberances were present along the hyphal walls (Figure 69). The irregularly thickened, nodose-septate hyphae were scattered unevenly throughout the thin-walled, nodose-septate hyphae and the fiber hyphae.

The fiber hyphae observed in the vegetative mycelium were similar to those found in agar culture and still culture. They were long, flexuous, 1.1-4.4 μ in diameter, refractive, and aseptate. The hyphal walls were colorless or yellowish-brown colored and up to 1.1 μ thick. The lumen was often reduced to a thin, granular appearing,

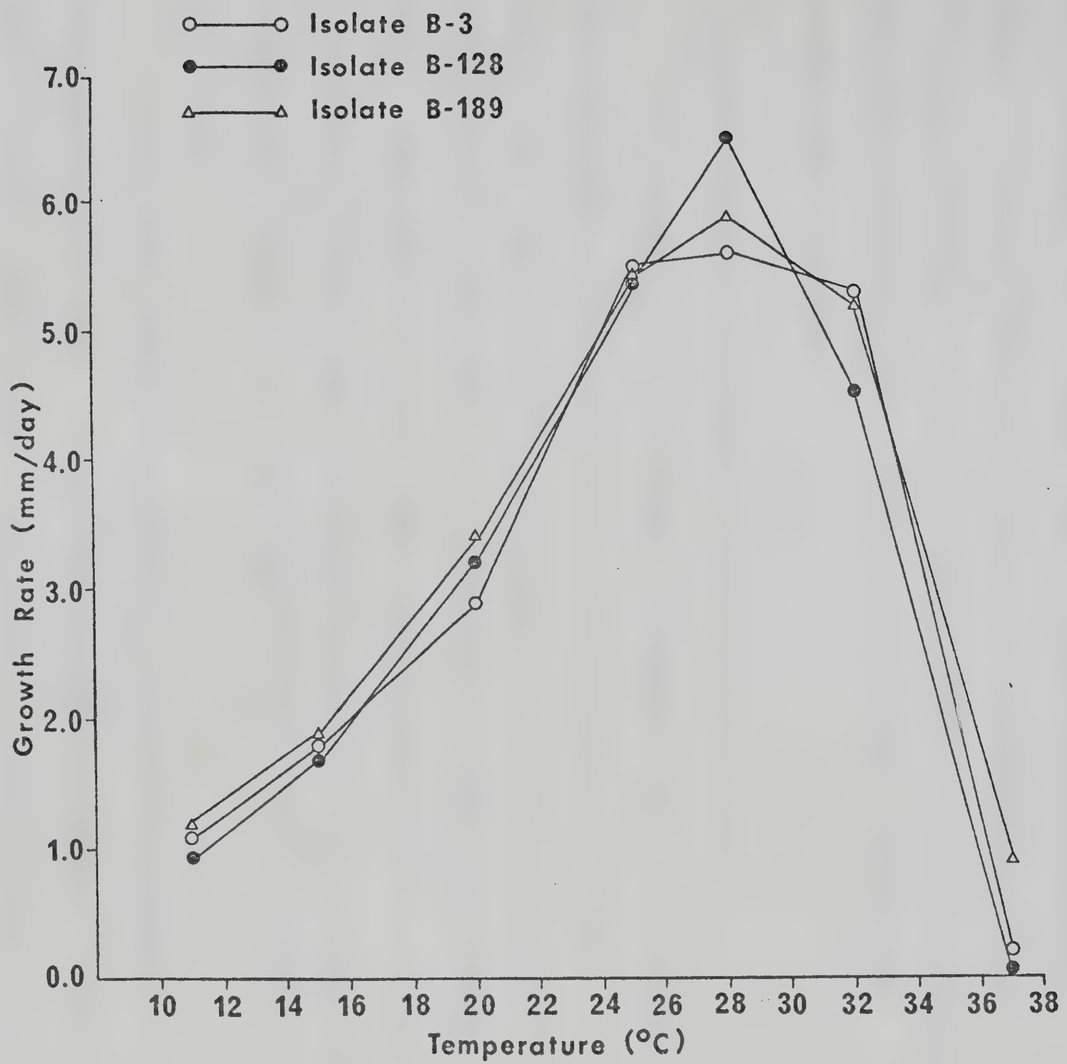
lightly stained line running down the center of the hypha. The apical and proximal portions of the hypha were occasionally thinner-walled and more deeply stained than the central portions. In most instances the apical portion was thickened right to the tip. Branching was infrequent with branch diameters being the same or slightly narrower than the main hypha. Two to four branches may arise from the apical portions of a hypha. The branches were often less than 50 µ in length. The fiber hyphae were densely intertangled and interwoven with each other (Figure 70) and the nodose-septate hyphae. They showed no organization.

In most of the wood-block cultures irregularly-shaped, variously-sized crystals were observed in the vegetative mycelium (Figure 71). The composition or identity of the crystals is not known. They did not dissolve in 5% aqueous KOH and they remained unstained in phloxine. These crystals were not observed in agar culture or still culture.

d. Temperature and growth of vegetative mycelium

The daily radial growth rates of *F. cajanderi* isolates B-3, B-128, and B-189 are shown in Figure 47. The greatest growth rate occurred at 28°C for all isolates, although the individual rates of growth varied between the isolates. Isolate B-3 showed the slowest growth rate of 5.6 mm/day compared to 5.9 mm/day for isolate B-189 and 6.5 mm/day for isolate B-128. The isolates varied in their ability to grow at 37°C. Isolate B-128 showed only trace growth at this temperature, while isolate B-3 grew 0.2 mm/day and isolate

Figure 47. Effect of temperature on the radial growth rate of the vegetative mycelium of *F. cajanderi*.


Isolate B-3
Isolate B-128
Isolate B-189
Growth Rate (mm/day)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Temperature (°C)

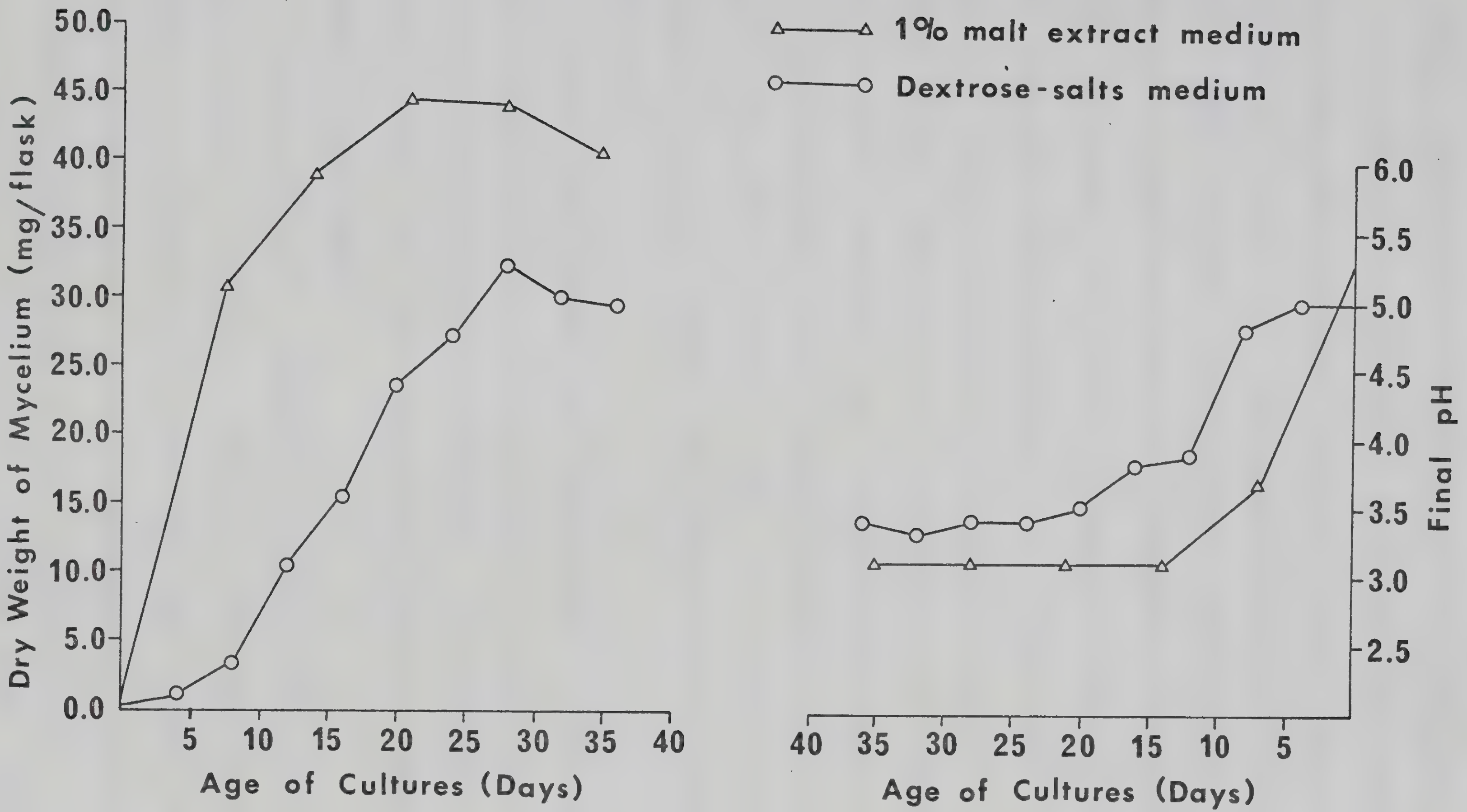
B-189 grew 0.9 mm/day. Trace growth of isolate B-128 was limited to a very fine, white mycelial covering of the inoculum plug. All three isolates showed similar growth at 11°C. Isolates B-3, B-128, and B-189 grew at the rate of 1.1 mm/day, 0.9 mm/day, and 1.2 mm/day respectively. It should be noted that agar cultures incubated at 11° and 15°C were difficult to measure due to the heavy condensation of moisture on the plate lids and the small amount of growth that occurred.

e. Growth-curve and carbon utilization studies

The growth of *F. cajanderi* (isolate B-3) in 1% malt extract medium and in dextrose-salts medium is shown in Figure 48. The results of the growth in 1% malt extract medium are the averages of 6 replicate flasks harvested every 7 days for 35 days. For the dextrose-salts medium, 5 replicate flasks were harvested every 4 days for 36 days. During each harvest, pH readings of the culture filtrates were taken.

In the malt extract medium, *F. cajanderi* showed no detectable lag phase. Very rapid growth occurred in the first 7 days. During the next 14 days, growth lessened considerably and after 21 days of growth maximum dry weight had occurred. Cultures older than 28 days showed an autolysis phase in which dry weight declined. During the 35 day growth period, the pH became more acid, dropping from an initial pH of 5.3 to a final pH of 3.1. The most rapid drop in pH occurred during the first 7 days of growth

Figure 48. Growth of *F. cajanderi* (isolate B-3) and pH changes in the medium in dextrose-salts medium and in 1% malt extract medium at 23°C.


1% malt extract medium
Dextrose-salts medium
Dry Weight of Mycelium (mg/flask)
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
5
10
15
20
25
30
35
40
Age of Cultures (Days)
Final pH
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
40
35
30
25
20
15
10
5
Age of Cultures (Days)

with the minimum pH occurring after 14 days of growth. No change of pH occurred during the autolysis phase.

The growth in the dextrose-salts medium showed a definite lag phase during the first 8 days. This was followed by a period of rapid growth during the next 20 days. After 28 days, maximum growth had occurred. Immediately after, an autolysis phase set in whereby dry weight decreased. The period of maximal growth in the dextrose-salts medium was very short, being only 2-3 days long. In the malt extract medium, maximal growth was maintained for approximately 7 days before an autolysis phase set in. The pH of the dextrose-salts medium decreased slightly during the first 8 days of growth and then dropped rapidly from pH 4.8 to 3.9 in the next 4 days. During the remaining period of growth, the pH gradually decreased to about 3.4.

Based on the growth of *F. cajanderi* in the dextrose-salts medium, an incubation period of 23 days was chosen for the carbon utilization studies. In this length of time, growth is well into the exponential phase and maximum growth is approached.

The growth of *F. cajanderi* (isolate B-3) on various carbon sources is shown in Table 2. The results are the averages of 5-7 replicate flasks for each carbon source. The hexose D-mannose and the polysaccharide cellobiose produced the best growth in terms of dry weight. Good growth occurred on dextrose and maltose while little growth

Table 2. Growth of *F. cajanderi* (isolate B-3) on various carbon sources.

Carbon Source	Oven-Dry Weight* (mg)	Final pH*
Dextrose	22.2	3.9
D-xylose	4.1	4.2
D-mannose	26.5	3.6
D-fructose	2.4	4.5
Galactose	5.5	4.2
Sucrose	3.4	4.5
Maltose	18.0	4.1
Cellobiose	27.7	3.7
No carbon (negative control)	0.7	4.8

* Results are the averages of 5-7 replicate flasks for each carbon source.

occurred on D-xylose, galactose, sucrose, and D-fructose. Negligible growth occurred in the negative controls in which no carbon source was added.

The pH of the medium in the carbon source flasks decreased as growth occurred. Generally, the greater the amount of mycelium produced, the lower the pH became as shown by the results of dextrose, D-mannose, and cellobiose. There was no pH change in the negative controls in which the initial pH after sterilization was 4.7-5.0 and the final pH 4.8.

f. Growth on wood-shavings with L-asparagine

The effect of L-asparagine on the decay of spruce sapwood shavings by two isolates of *F. cajanderi* is shown in Table 3. The weight losses were calculated as percentages of the initial oven-dry weight of the shavings and are based on the average of 5 replicate cultures at each L-asparagine concentration. The cultures were incubated for 12 months before weight losses were measured. In both isolates the largest weight loss occurred in cultures which contained little or no added L-asparagine. At concentrations higher than 0.1%, the rate of decay was markedly reduced compared to the controls. The weight losses in control cultures in which no L-asparagine was added were virtually identical in both isolates. However, at concentrations of 0.1% L-asparagine isolate B-3 showed a slight increase in weight loss while isolate B-128 showed a slight decrease in weight loss compared to the

Table 3. Effect of L-asparagine on the decay of spruce shavings by *F. cajanderi* (isolates B-3 and B-128).

Concentration of L-asparagine added (%)	Loss in dry weight as percentage of initial weight*	
	B-3	B-128
0.0	11.23	11.82
0.1	12.05	8.13
0.5	1.19**	1.83
1.0	1.44	1.71
2.0	1.56	2.11

* Each percentage represents the average of 5 replicate cultures.

** Average of 4 replicate cultures only.

controls. At concentrations of 0.5%, 1.0%, and 2.0 % L-asparagine, weight losses were similar in both isolates. Weight losses ranged from 1.19% for isolate B-3 to 2.11% for isolate B-128.

The macroscopic appearance of the wood-shavings substantiated the greater decay rate in the control cultures and those containing 0.1% L-asparagine. In these cultures heavy mycelial growth was observed. Along the top edges of the culture dishes dense, felty, light to dark-brown mats of mycelium had formed. The surface shavings were heavily covered with white, cottony mycelium while the lower shavings were completely infiltrated by very fine, white mycelium. The color of the wood-shavings ranged from cream-white, slightly yellow to light-brown. Cultures with heavy mycelial growth frequently showed a number of reddish stained areas in the wood-shavings. These stained areas were irregular in size and shape and were usually observed in the lower half of the culture dishes. A culture dish may have as many as 7-10 stained areas in the wood-shavings.

Almost no mycelial growth was observed in cultures containing concentrations of L-asparagine higher than 0.1%. Very sparse, white mycelium was seen in the area of the inoculum plug but the rest of the wood-shavings showed no visible mycelial growth. Stained areas were also absent in these cultures. The color of the wood-shavings in all culture dishes containing L-asparagine was yellowish compared to the cream-white wood-shavings of the

contamination controls.

2. Development of the basidiocarp

For each of the three types of cultures the development and structure of the basidiocarps will be described in two sections: macroscopic characters and microscopic characters. Nobles' terms are used in the descriptions of the primordia and basidiocarps. The first stage of basidiocarp development is the primordium which is considered here to be the compact, felty, light-pink to light-brown, raised mycelial area that usually forms near the edges of the cultures. The basidiocarps are the basidium-bearing, poroid structures that develop from the primordial areas.

a. Agar culture - Macroscopic characters

Cultures of *F. cajanderi* readily fruited on malt agar plates when incubated at room temperature near a south-facing window. Thirty-three isolates were examined for fruiting and of these, twenty-seven fruited within 5 weeks. Although the length of time for fruiting varied among isolates, most fruited in 3-4 weeks. The basidiocarps generally developed along the margin of the mycelial mat within 2 cm of the Petri plate edge or occasionally, right on the sides of the plate. Infrequently, fruiting occurred on the inoculum plug or in scattered areas of the central portions of the culture. The first indication of fruiting is the formation of primordia which are dense, compact,

felty, raised mycelial areas. The primordia may be light-pink, reddish-pink, or light-brown colored. They may form in scattered locations along the culture edge or may completely encircle the central portions of the culture (Figure 50). Within 2-3 days pores begin to develop in the compact, felty primordial areas (Figures 72, 73). The pores develop in various locations on the primordial surface and, in most cultures, only a small area of the primordial surface develops pores. In the first stage of pore development shallow furrows and ridges form on the dense, felty mat. The furrowed, ridged areas become more distinct and sharper in outline within one or two days and can soon be recognized as pore walls. The pores initially develop at the very edge of the mycelial mat and progressively form new pores to the inside. As the pore walls increase in depth the pore mouths begin to take shape. The pore mouths are not regular in shape but are of various forms due to the different thicknesses and configurations of the pore walls. The pore mouths may be rounded, sub-angular, or daedaloid in form (Figures 74, 75). The depth of the fully-developed pores ranges from less than 0.5 mm to 1.5 mm. The pore walls are firm, compact, very coherent, and light-pink or reddish-pink colored. Cream-white basidiospore deposits are observed on the Petri plate lids 1-3 days after the first pores have formed.

Microscopic characters

The compact, felty primordial areas are composed of

nodose-septate hyphae and fiber hyphae. The nodose-septate hyphae are thin-walled, but, in older cultures they become irregularly thickened. The thin-walled, nodose-septate hyphae are abundant in the primordial areas, often forming over half of the mycelium. These hyphae are frequently branched, abundantly clamped, 1.1-2.8 μ in diameter, and hyaline when unstained. In phloxine the hyphal contents stain deeply and appear dense and homogeneous. The hyphal walls are thin throughout and show no signs of wall thickening. Branching occurs near clamp connections or rarely, directly from a clamp connection. The branches are the same diameter or slightly narrower than the main hypha. There was no organization of the thin-walled, nodose-septate hyphae. They were densely intertangled and interwoven with the fiber hyphae.

The fiber hyphae in the primordial areas are similar in form and appearance to the fiber hyphae of the aerial mycelium in the central portions of the mat. The hyphal walls are colorless or yellowish-brown and are about 1 μ thick. Often the hyphal walls completely occlude the lumen which is narrow, granular appearing, and lightly stained with phloxine. In the apical and proximal ends of the fiber hyphae the walls are thinner and the lumina are wider and more deeply stained. Developing fiber hyphae are often seen in the primordial areas. Their lengths vary according to their stage of development: fully developed fiber hyphae have measured over 1200 μ in length. The fiber hyphae

develop from the thin-walled, nodose-septate hyphae in the same manner as the fiber hyphae of the central portions of the mat. Branching occurs in the apical and central portions of a fiber hypha and branch diameters are the same or slightly narrower than the main hypha. The very coherent and compact nature of the primordial areas is due to the dense intertangling and interweaving of the fiber hyphae.

In the pore areas the pore walls are composed of intertangled fiber hyphae which grow in a downward direction. The fiber hyphae are not arranged in parallel alignment but interweave with each other in all directions. The leading edge of the pore walls is composed of flexuous fiber hyphae whose apical ends grow in a downward direction (Figure 76). The apical ends of these fiber hyphae are deeply stained, thin-walled, and unbranched. Basidia line the walls of the pores in a palisade layer. They are clavate-shaped, deeply stained with phloxine, 7.7-20.4 x 3.3-6.6 μ in size, thin-walled, and clamped at their base (Figure 77). Four slender, 2.8-3.3 μ long sterigmata are occasionally observed on the basidia. The basidiospores formed in culture are indistinguishable from those observed in natural basidiocarps. They are narrow-cylindric, smooth-walled, slightly curved at one end, and 4.0-7.0 x 1.5-1.9 (-2.3) μ in size and hyaline when unstained. The basidia arise as terminal cells of the thin-walled, nodose-septate hyphae that are found in dense

masses along the edges of the pore walls. The interior of the pore walls contain scattered masses of nodose-septate hyphae. The basidia develop very soon after pore wall formation has started. Pore walls that are approximately 200 μ in depth have been observed with basidia lining the basal portions of the walls. When the pore walls have reached their maximum depth (approximately 1.5 mm), the basidia line the entire inner wall surfaces except for the leading edge where the fiber hyphae protrude. The thin-walled, nodose-septate hyphae in the pore walls become irregularly thickened in old cultures (usually 5 or more weeks old). The irregularly thickened, nodose-septate hyphae develop in the same manner as those of the aerial mycelium in the central portions of the mat. The appearance and form of the irregularly thickened hyphae is also similar. Occasionally in the old cultures sterile basidia are attached to the irregularly thickened, nodose-septate hyphae.

b. Still culture - Macroscopic characters

Basidiocarp development in still culture was erratic. Most cultures produced good growth of vegetative mycelium and numerous primordia but pore formation rarely occurred. Over fifty still cultures were examined for fruiting, but only three cultures showed pore development. In most cultures 1/3 to 1/2 of the medium surface is covered by aerial mats after 25 days. Primordial development begins about 21 days after inoculation. The primordia are dense,

compact, felty, hemispherical mycelial masses. They are white at first but as they increase in size they become light-pink, light-brown, or medium-brown. The number of primordia that forms in each culture varies. A culture may contain from 1 to over 30 primordia, although the usual number is 5-10 primordia. The primordia vary in size depending on their stage of development. Initially, they are small, compact, white, circular masses about 1 mm across (Figure 78). These circular masses gradually increase in size and become hemispherical in shape, reaching a maximum size of 5-8 mm in about 2 weeks (Figure 79). All primordia in a culture do not begin development at the same time. Different stages of primordial development are often observed in an individual culture with small primordia developing among the large primordia (Figure 78). The primordia usually form at the margins of the aerial mats (Figure 80) or occasionally away from the mats as isolated mycelial masses (Figure 81). In some instances, they develop in scattered areas over the entire surface of the aerial mat (Figure 82). Primordia developing along the margin of the aerial mat occasionally become confluent as they increase in size. The result is a raised mycelial ridge that partly encircles the central portion of the aerial mat.

As noted earlier, the great majority of the still cultures did not develop beyond the primordial stage. In the three pore-forming cultures basidiocarp development

differed slightly from that observed in agar culture. Pore development in still culture was much slower than in agar culture. In still culture pore formation occurred about 3 months after inoculation. The primordial surface becomes furrowed and ridged and after 7-10 days recognizable pore walls have formed. The pore walls develop by upward growth of the mycelium from the primordial surface. The walls vary in thickness and configuration and the resulting pore mouths are rounded or somewhat daedaloid in form. The pore depth is about 1 mm and the walls are pink or light-brown, firm, and compact appearing. In one of the pore-forming cultures the basidiocarp developed along a mycelial ridge that partly encircled the central portion of the mat (Figure 83). Pores also developed in scattered areas of the primordial surface giving the mycelial mass a pitted appearance (Figure 84).

Microscopic characters

The small developing primordial masses that have formed approximately 21 days after inoculation are composed of thin-walled, nodose-septate hyphae and fiber hyphae. The contents of the thin-walled hyphae are dense, granular appearing, homogeneous, and deeply stained with phloxine. The hyphal walls are thin throughout except for the occasional swollen, solid appearing clamp connection or short, solid appearing protuberance. The hyphae are abundantly clamped, occasionally branched, and 1.1-3.3 μ in diameter. The branches arise along all parts of the hyphae

with branch diameters the same or slightly narrower than the main hypha. The nodose-septate hyphae occasionally bear long, flexuous, thin-walled, aseptate, terminal cells. These cells are deeply stained and are the first stage of fiber hyphal formation. Their development into a fiber hypha has already been described in the vegetative features of still culture. The nodose-septate hyphae form approximately 1/4 of the mycelium in the primordia. They are distributed fairly evenly throughout the primordia among the fiber hyphae.

In the developing primordia the fiber hyphae form approximately 3/4 of the mycelium. These hyphae are similar in form and appearance to the fiber hyphae of the vegetative mycelium. They are long, flexuous, 1.1-4.4 μ in diameter, aseptate, and have thick, colorless or slightly yellowish-brown, refractive walls. The lumen is often narrow and very lightly stained. Branching occasionally occurs, often in the apical and central portions of the hypha where from 1-6 branches may form along a short length of the hypha. In the apical and proximal portions of the fiber hyphae the walls are often thin, and the lumina wider and more deeply stained than the central portions. These deeply stained hyphae are developing fiber hyphae which have arisen from the thin-walled, nodose-septate hyphae. The apical growth of the developing fiber hyphae and the formation of new fiber hyphae by the nodose-septate hyphae gradually increases the mass and size of the primordium. The densely

intertangled and interwoven fiber hyphae are responsible for the compact, coherent nature of the primordium.

In the 3 month-old cultures in which the primordia have not developed pores, the nodose-septate hyphae and fiber hyphae are again observed. However, most of the nodose-septate hyphae are now irregularly thickened. Approximately 1/4-1/2 of the primordial mass is composed of nodose-septate hyphae. The irregularly thickened hyphae are similar to the irregularly thickened, nodose-septate hyphae of the vegetative mycelium. They are frequently clamped, occasionally branched, and 1.1-4.4 μ in diameter. The lumen is narrow, sinuous, deeply stained, and sometimes is completely occluded. The hyphal walls are of variable thickness and are colorless and refractive. In some instances thin-walled, nodose-septate hyphae are observed, but they often show swollen clamp connections and short protuberances indicating the onset of wall thickening. As in the small, developing primordial masses, the nodose-septate hyphae are fairly evenly distributed throughout the primordial mass. Developing basidia have not been observed in these primordia, although very rarely a thin-walled chlamydospore is seen.

The fiber hyphae in the primordia are also similar in form to the fiber hyphae of the vegetative mycelium. They are long, flexuous, aseptate, and 1.7-4.4 μ in diameter. The hyphal walls are thick, refractive and yellowish-brown or light-brown. The colored hyphal walls are responsible

for the light-pink, light-brown, or medium-brown appearance of the primordia. The walls occasionally completely occlude the lumen which is narrow, granular appearing, and very lightly stained in the central portions of the hyphae. The apical and proximal portions of the hypha are thinner-walled than the central portions, but the lumen is granular appearing and lightly stained, similar to the fully developed fiber hyphae of the vegetative mycelium. Branching occurs in the central and apical portions of the hypha with branches measuring up to 150 μ in length, although they are often less than 50 μ. The branches are the same diameter or slightly narrower than the hypha from which they arose. There may be from 1-5, or rarely 6 branches arising along a short length of a hypha. Hyphal lengths were difficult to measure since the fully developed fiber hyphae were rarely seen attached to the nodose-septate hyphae. Fragments of hyphae have measured well over 1000 μ in length. The hyphal organization in the primordia was the same as before. The fiber hyphae were densely intertangled and interwoven together maintaining the compact, coherent nature of the primordia.

In the three fruiting cultures, the primordia were composed of nodose-septate hyphae and fiber hyphae which were similar to those found in the non-fruiting cultures. The nodose-septate hyphae are occasionally branched, frequently clamped, deeply stained with phloxine, and 1.1-3.3 μ in diameter. They are distributed evenly throughout

the primordial mass or infrequently in intertangled masses among the fiber hyphae. Approximately 3 months after inoculation pore walls began to form on the primordia. They developed in the same manner as the pores in agar culture, but in still culture the walls formed by upward growth of the mycelial surface. The developing pore walls are composed of densely interwoven fiber hyphae which are occasionally deeply stained in the apical and proximal portions. The leading edge of the pore wall consists of deeply stained, apical ends of fiber hyphae which are often unbranched. These deeply stained hyphae are developing fiber hyphae which extend the length of the pore wall by apical growth. The edge of the pore wall continues upward growth until the pores are approximately 1 mm deep. The interior of the pore walls is composed mainly of densely interwoven fiber hyphae which are often branched in the apical and central portions. Between 1-6 branches may develop along a short length of the fiber hypha. The branches are usually less than 100 µ in length and are the same diameter or slightly narrower than the hypha from which they arose.

The great majority of the nodose-septate hyphae in the pore walls are thin-walled, unlike the nodose-septate hyphae of the non-fruiting cultures which had become irregularly thickened. The nodose-septate hyphae in the interior of the pore wall are evenly distributed among the fiber hyphae, but along the pore walls are masses of

densely intertangled nodose-septate hyphae. These hyphae are thin-walled throughout and show no indication of wall thickening. They are frequently clamped, profusely branched, granular appearing, deeply stained with phloxine, and 1.1-2.8 μ in diameter. The hyphae are often contorted and tortuous in form and basidia are often observed along their length developing as terminal cells. The basidia initially develop at the base of the pores, often before the pore walls have reached their maximum length. The basidia are clavate-shaped, thin-walled, deeply stained with phloxine, and 9.9-15.4 x 4.4-5.6 μ in size. They are infrequently observed with 4 immature basidiospores developing on slender, 3 μ long sterigmata (Figure 88). Basidia develop along most of the pore wall except at the leading edge where the fiber hyphae protrude. Although numerous basidia were formed, basidiospores were infrequently observed. This may be due to their deposition towards the base of the pores where it would be difficult to distinguish the basidiospores among the basidia and hyphae. The basidiospores observed were indistinguishable from those formed in agar culture. They are narrow-cylindric, slightly curved, deeply stained with phloxine, smooth-walled, and 4.0-7.0 x 1.5-2.0 (-2.3) μ in size. The basidiospores are hyaline when unstained.

The fiber hyphae at the leading edge of the fully developed pore walls are no longer deeply stained at their apical portions, but now appear granular and lightly

stained (Figure 85). The lumen is often wider at the apical portion (Figure 86), but further back the hyphal walls are thicker and the lumen narrow and lightly stained. The hyphal walls are refractive, yellowish-brown or colorless, and are approximately 1 µ thick in the central portions of the hypha. The fiber hyphae occasionally measure over 1000 µ in length, but it was often difficult to measure intact hyphae since they had separated from the nodose-septate hyphae. The hyphae were very densely intertangled and interwoven in the pore walls (Figure 87) which accounted for the firm, compact pore walls.

c. Wood-block culture - Macroscopic characters

Since the primordia and basidiocarps that formed at room temperature were as variable in appearance as those formed at 20°C, the development of these structures will be described together. The great majority of cultures exhibited some primordial formation by the end of the 12 month incubation period. Of the 36 cultures examined only one did not show any primordial development. The formation of pores was almost exclusively restricted to cultures of isolate B-128. Ten of the 12 cultures of isolate B-128 developed pores, while only one culture of isolate B-3 developed pores. None of the cultures of isolate B-189 developed pores, although numerous primordia had formed.

The primordia were variable in size and appearance and no correlation was noted between the substrate type, the incubation temperature, or the isolate used. Primordia

formation began approximately 2-3 months after inoculation and continued at various times throughout the incubation period. By the end of 8 months many cultures contained so many primordia that it was difficult to discern the formation of new primordia. Cultures frequently contained primordia of various sizes indicating their different times and rates of development. In general, the primordia were dense, compact, felty, hemispherical masses that usually formed along the top surface and top edges of the wood blocks (Figures 66, 89, 90). Occasionally, primordia formed along the sides of the blocks. The primordia often became confluent as they increased in size resulting in an uneven, hummocky mycelial mass on the block surface (Figure 91). Individual primordia ranged in size from 1 mm to 10 mm across, although the usual size was 2-5 mm across and 2-3 mm in height. The number of primordia developing on a block varied from less than 5 to more than 30 primordia. The confluent primordia were often difficult to distinguish as individual structures. The primordia initially developed as small (approximately 1 mm across), compact, white mycelial masses that formed among the vegetative mycelium. As the primordia increased in size they became hemispherical in form and often pink colored. In some cultures the primordia remained white even though they reached 5 mm or more across. The pink primordia gradually became light-brown to medium-brown in most cultures.

Pore formation occurred in the pink or light-brown,

hemispherical primordia in the same manner as that observed in still culture. The surface of the primordium became furrowed and ridged as a result of upward growth of the mycelial surface (Figure 92). Within a few days the pore walls and pore mouths could be distinguished. The pore mouths were daedaloid to somewhat rounded in shape (Figures 93, 94, 95) and 1-2 mm in depth. The rounded pore mouths were 1-3 mm across, and when widely separated on the mycelial surface, gave the surface a pitted appearance. The walls of the pores were very coherent and compact, similar to that of the pores formed in agar culture and still culture. In some of the cultures pores had formed along the sides of the wood block (Figure 96), or down the sides of the primordia that had formed on the edges of the block (Figure 97). These pores were elongated and open and appeared as parallel furrows and ridges on the mycelial surface. The walls of the elongate pores were coherent and compact and were approximately 1 mm thick. The pore walls were spaced at various distances apart and were occasionally incomplete as shown in Figure 97.

Microscopic characters

The hemispherical primordia were composed of nodose-septate hyphae and fiber hyphae that were similar in appearance to the hyphae observed in agar culture and still culture. The nodose-septate hyphae, which formed 1/4 to 1/2 of the mycelium, were distributed fairly evenly throughout the primordial mass. Occasionally, densely intertangled

masses or "islands" of nodose-septate hyphae were observed among the fiber hyphae. The nodose-septate hyphae in the primordia were thin-walled or irregularly thickened. The relative amounts of each hyphal type varied between the individual cultures. In some instances, the thin-walled hyphae had swollen clamp connections and solid appearing, short protuberances indicating the beginning of irregular wall thickening. The contents of the thin-walled, nodose-septate hyphae were homogeneous, granular appearing, and deeply stained with phloxine. The hyphae were hyaline when unstained and 1.1-3.3 μ in diameter. Branching often occurred with branch diameters being the same or slightly narrower than the main hypha. The irregularly thickened, nodose-septate hyphae were of the same form and diameter as the thin-walled hyphae. They differed in the presence of swollen, solid appearing clamp connections, solid appearing, short protuberances, and a narrow, sinuous lumen in the irregularly thickened hyphae. These hyphae readily separated from each other leaving one end of the hypha with the hook portion of the clamp (Figure 98).

The fiber hyphae were long, flexuous, sparingly branched, and 1.7-4.4 μ in diameter. The hyphal walls were colorless or yellowish-brown, refractive, and approximately 1 μ thick, although the lumina in the central portions of the hyphae frequently appeared completely occluded. The fiber hyphae were often fully developed, since the lumina were lightly stained and the only thin-walled portions were the

very extremities of the hyphae. The hyphae often measured over 1000 μ in length. In rare instances, a developing fiber hypha was observed with deeply stained contents and thin walls. The great preponderance of fully developed fiber hyphae suggests that future pore development in the 12 month-old wood-block culture primordia is unlikely. This situation is similar to the 3 month-old non-fruiting still cultures in which the primordia were composed mainly of fully developed fiber hyphae. The fiber hyphae in the wood-block culture primordia were compactly intertangled and interwoven with numerous hyphal ends protruding outward from the edge of the mycelial mass (Figure 99). The interwoven fiber hyphae are responsible for the dense, coherent nature of the primordia.

In the daedaloid and rounded pore areas, the fiber hyphae in the walls were very densely intertangled and interwoven in the same manner as the hyphae in the still culture pore walls. The leading edges of the pore walls were composed of protruding, unbranched, apical ends of fully developed fiber hyphae that had grown in an upward direction. Along the pore walls were scattered masses of densely intertangled thin-walled, nodose-septate hyphae. Immature, clavate basidia were often seen arising from these hyphae, but very few basidia with sterigmata or basidiospores were observed. The basidia measured 11.2-19.8 x 5.0-5.5 μ, while the slender sterigmata were approximately 3 μ in length. The basidia did not line the entire pore wall

surface but were found in discontinuous areas along the pore walls. Mature basidiospores were very infrequently observed in the pores. It appears that the basidiospores that were deposited in the upward facing pores had broken down since masses of granular, lightly stained debris were observed at the base of the pores. The debris may also be from basidia that had formed earlier in pore development and had now broken down. The few intact basidiospores observed were identical to those formed in agar culture and still culture. In many of the pores numerous, irregularly-shaped, unstained crystals were observed. These crystals were identical to those found in the vegetative mycelium.

The elongate, open pores were composed mainly of fiber hyphae that were very densely intertangled and interwoven. The edges of the pore walls consisted of unbranched, lightly stained ends of fiber hyphae that protruded outward in a horizontal manner (Figure 100). The hyphae observed in the elongate pores were identical to those found in the daedaloid and round pores. The fiber hyphae were long, flexuous, aseptate, and 1.7-4.4 μ in diameter. The lumen was narrow, granular appearing, and lightly stained. The hyphal walls were colorless or yellowish-brown, refractive, and approximately 1 μ thick. Branching occurred sparingly, usually in the apical portions of the hyphae, or occasionally in the central portions. The branches were frequently less than 100 μ in length and were

the same diameter or slightly narrower than the hypha from which they arose.

The thin-walled, nodose-septate hyphae were distributed throughout the interior of the elongate pore walls, and scattered masses of densely intertangled, nodose-septate hyphae were observed along the pore wall surfaces. Immature, clavate basidia were often seen developing in a palisade layer from the intertangled masses of hyphae, but few fertile basidia were observed. Along certain areas of the pore walls were masses of lightly stained, granular debris that appeared to be basidia that had broken down. The nodose-septate hyphae within the pore walls and in the intertangled masses were granular appearing, homogeneous, abundantly clamped, and 1.7-3.3 μ in diameter. They stained deeply with phloxine and were thin-walled throughout. The nodose-septate hyphae in the intertangled masses were profusely branched and somewhat contorted while the hyphae in the pore wall interior were less frequently branched and flexuous.

Figure 49. Malt agar culture of *F. cajanderi* (B-128), 24 days old, grown at room temperature. Note the cottony-floccose texture of the central portions and the slight development of compact, felty mycelium at the plate edge. X0.70.

Figure 50. Malt agar culture of *F. cajanderi* (B-3), 24 days old, grown at room temperature. Note the dense, compact, felty mycelium at the plate edge. X0.70.

Figure 51. Fiber hypha from the aerial mycelium of agar culture. Note the thick walls, narrow lumen, and the lightly stained contents. X1480.

Figure 52. Branching occurring at the apical portion of a fiber hypha from the aerial mycelium. X580.

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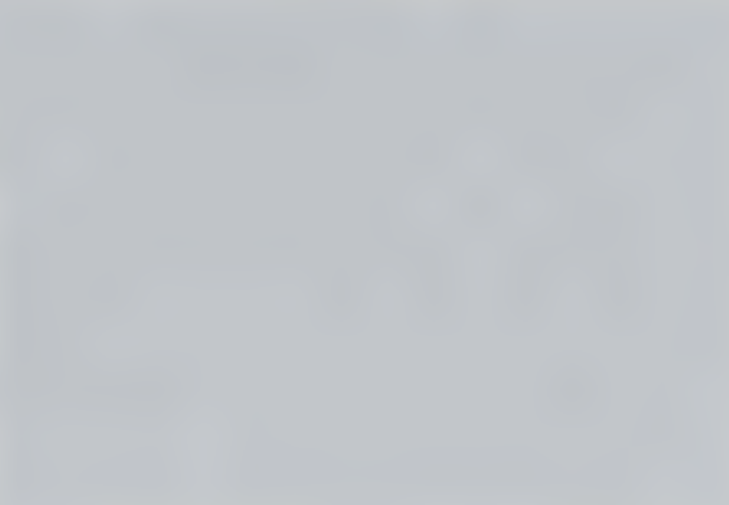


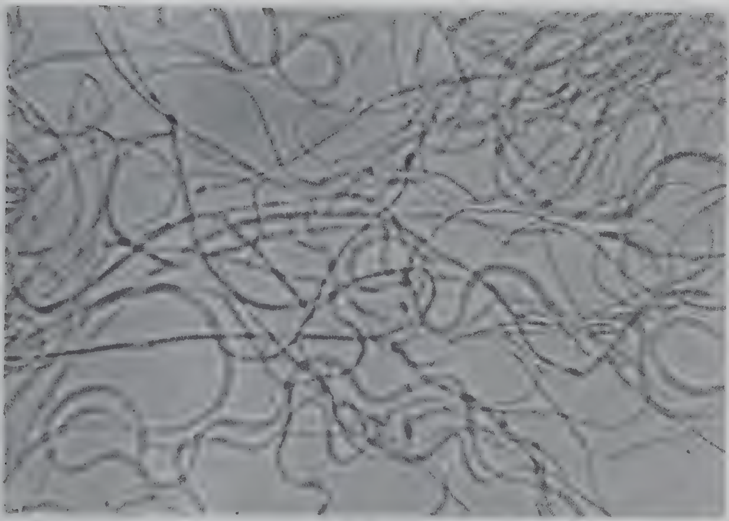
Figure 53. Long, flexuous fiber hyphae and nodose-septate hyphae that form the aerial mycelium. X580.

Figure 54. Irregularly thickened, nodose-septate hyphae from the aerial mycelium. Note the narrow, sinuous lumina and the broken clamp connection (arrow). X695.

Figure 55. Irregularly thickened, nodose-septate hyphae from the aerial mycelium. Note the narrow, sinuous lumina and the thick walls. X1815.

Figure 56. Thin-walled, deeply stained nodose-septate hyphae from the submerged mycelium. X580.

Figure 57. Thin-walled, deeply stained chlamydospore from the submerged mycelium. X1480.



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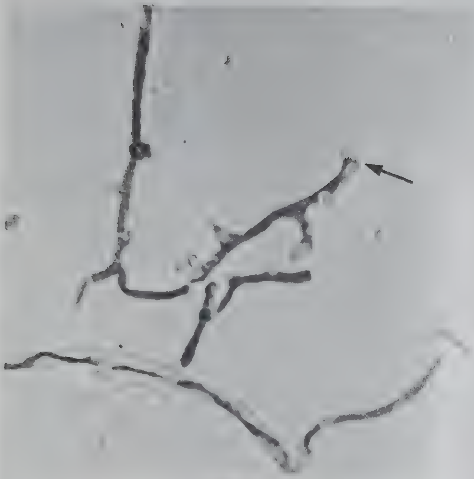

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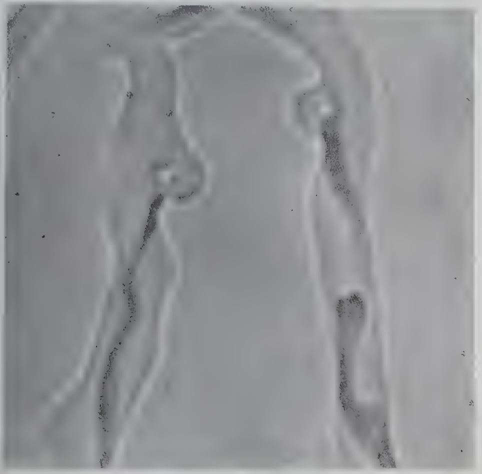

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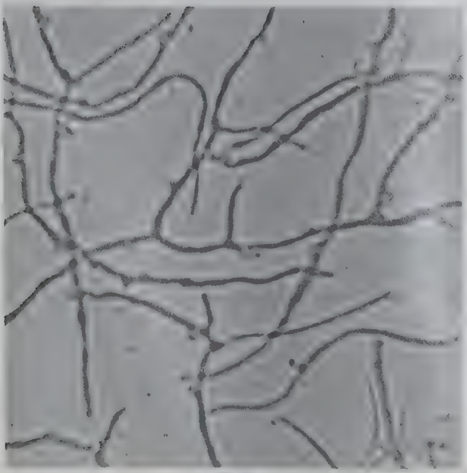

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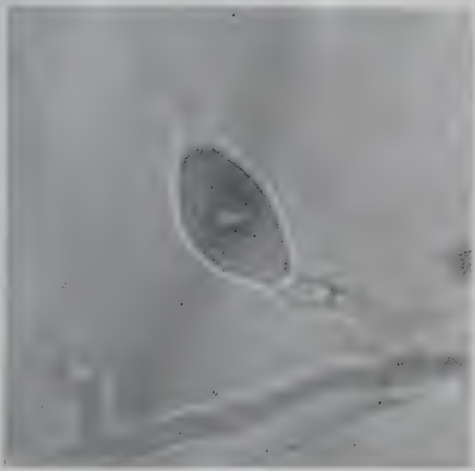

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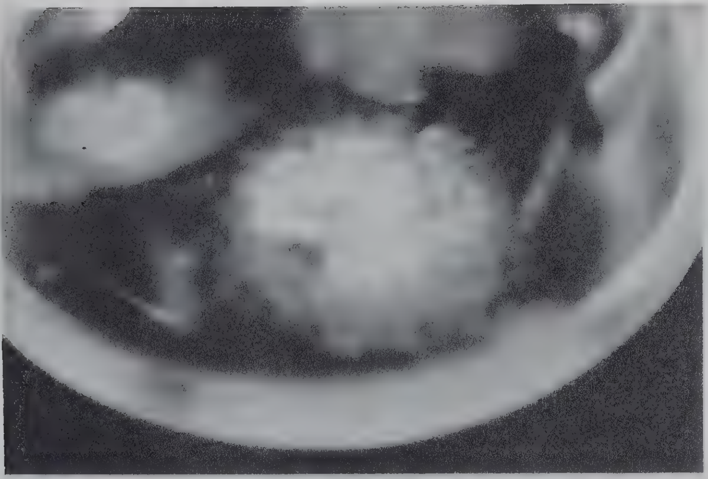
Figure 58. Still culture of *F. cajanderi* (B-3), 11 days old, grown at room temperature. Note the white, powdery aerial mycelium and the irregular, sparse margin. X1.6.

Figure 59. Still culture of *F. cajanderi* (B-128), 30 days old, grown at room temperature. Note the white, cottony-floccose mycelium covering about half of the medium surface. X1.3.

Figure 60. Thin-walled, nodose-septate hyphae from the submerged mycelium of still culture. Note the branch forming directly from a clamp connection. X695.



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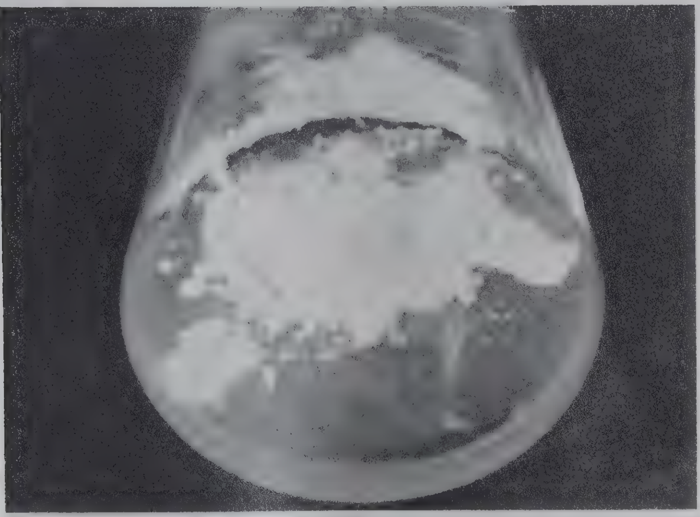

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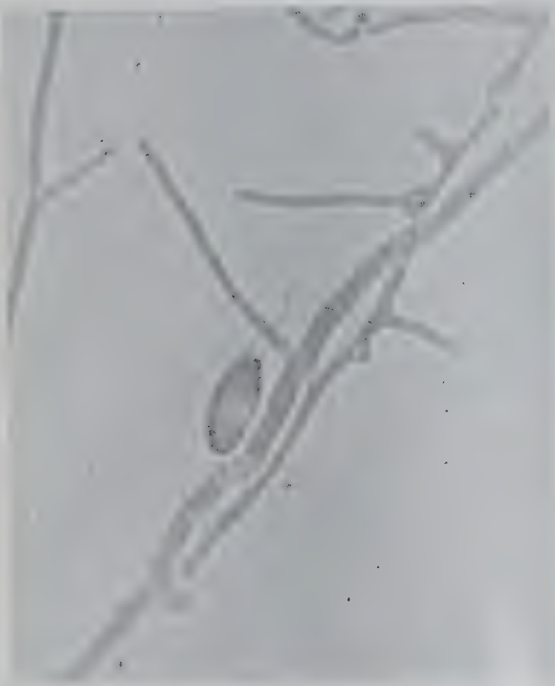
Figure 61. Thin-walled, granular appearing, ellipsoidal chlamydospore from the submerged mycelium. X695.

Figure 62. Elongate, thin-walled chlamydospores from the submerged mycelium. Note the simple septa on each side of the chlamydospores. X695.

Figure 63. Branched apical portion of a fiber hypha from the aerial mycelium. X580.

Figure 64. Deeply stained, thin-walled apical end of a developing fiber hypha from the aerial mycelium. X1480.



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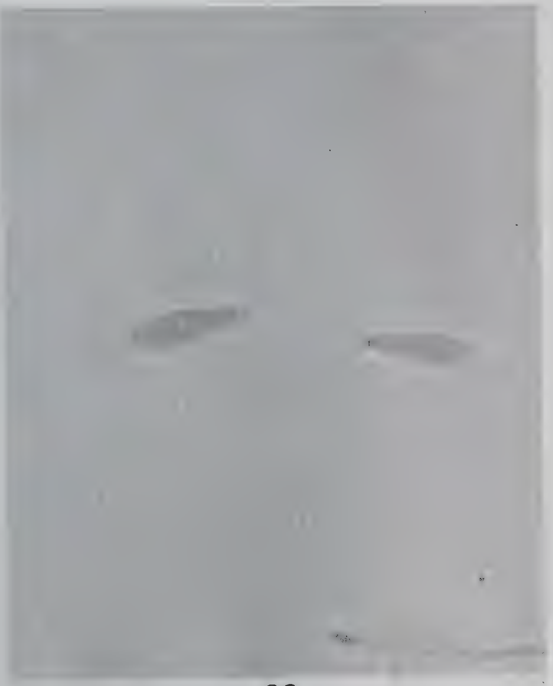

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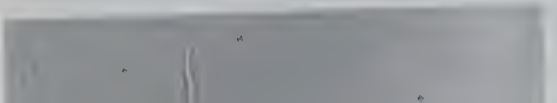

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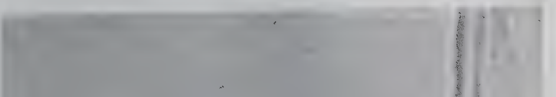

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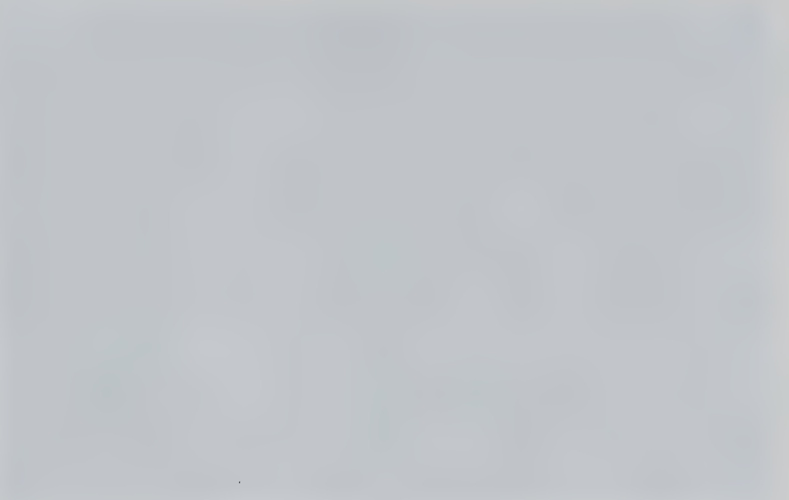


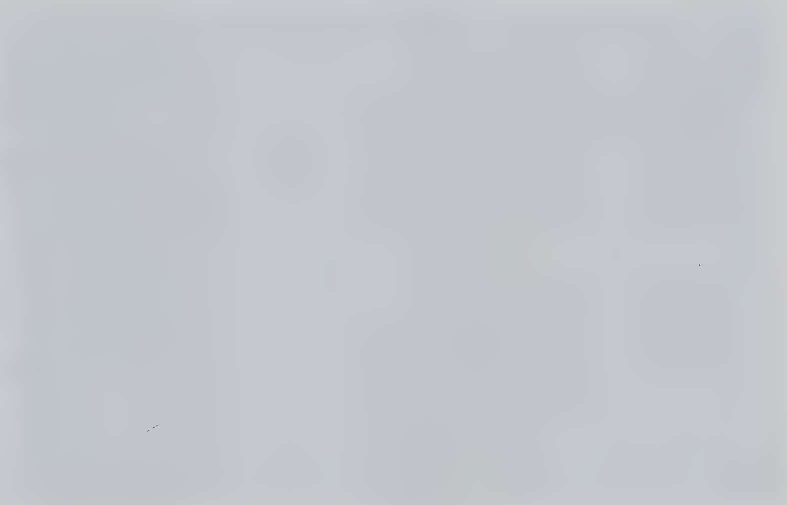


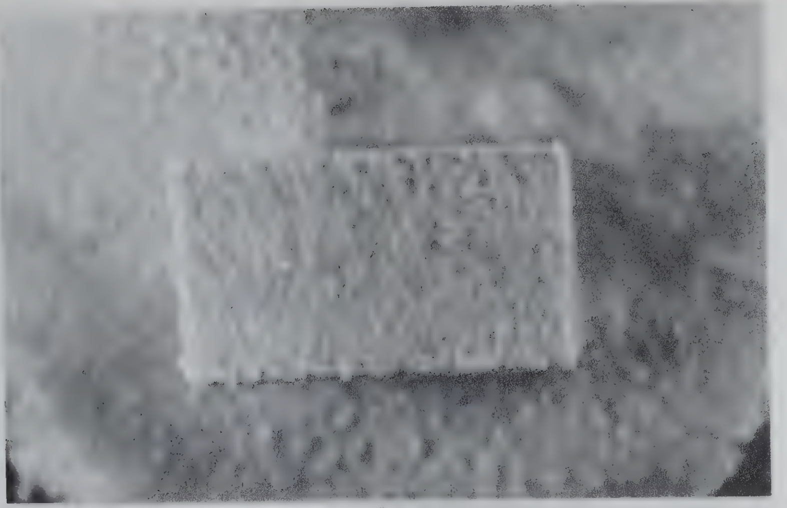
Figure 65. Pine block culture of *F. cajanderi* (B-189) grown at room temperature for 12 months. Note the dense, compact vegetative mycelium covering the top surface of the block. X1.6.

Figures 66 and 67.

Pine block culture of *F. cajanderi* (B-189) grown at 20°C for 10 months.

Top view - note the sparse, cottony-floccose vegetative mycelium and the small, hemispherical primordia forming at the top edge of the block. X1.3.

Side view - note the dense, cottony-floccose vegetative mycelium covering the side of the block. X1.7.



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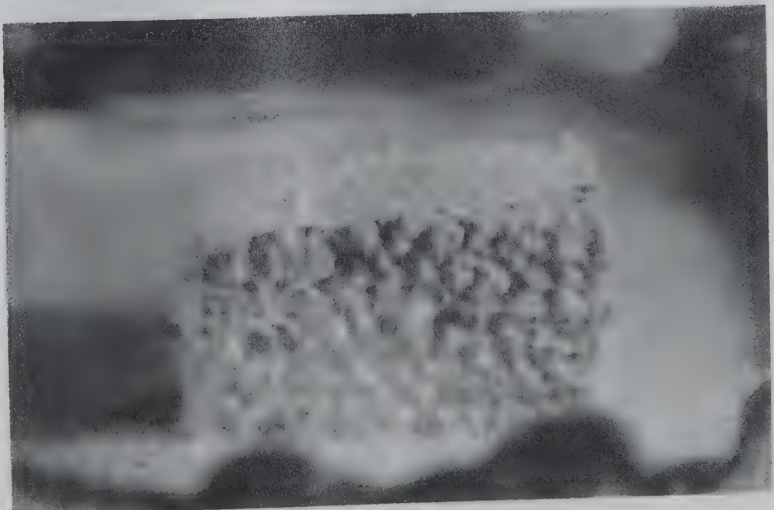

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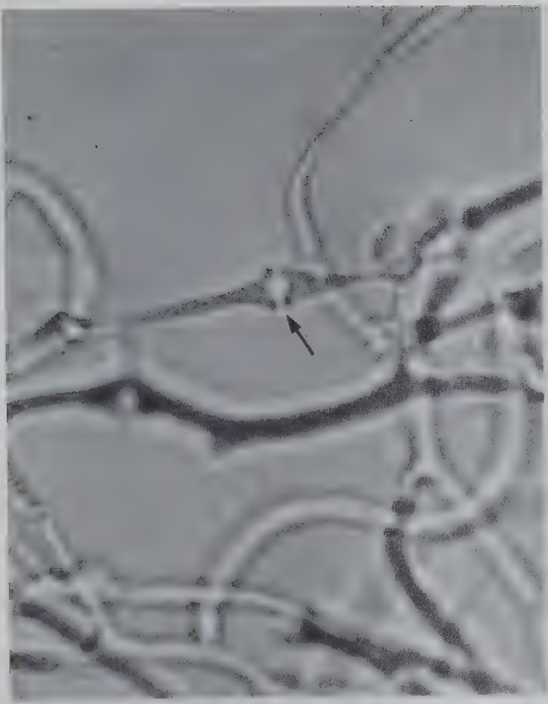
Figure 68. Thin-walled, nodose-septate hypha with "medallion" type of clamp connection (arrow). X580.

Figure 69. Irregularly thickened, nodose-septate hypha with short, solid protuberances along the hyphal wall (arrows). X580.

Figure 70. Densely intertangled and interwoven fiber hyphae of the vegetative mycelium. X580.

Figure 71. Irregularly-shaped crystals in the vegetative mycelium of an aspen poplar block culture. X180.



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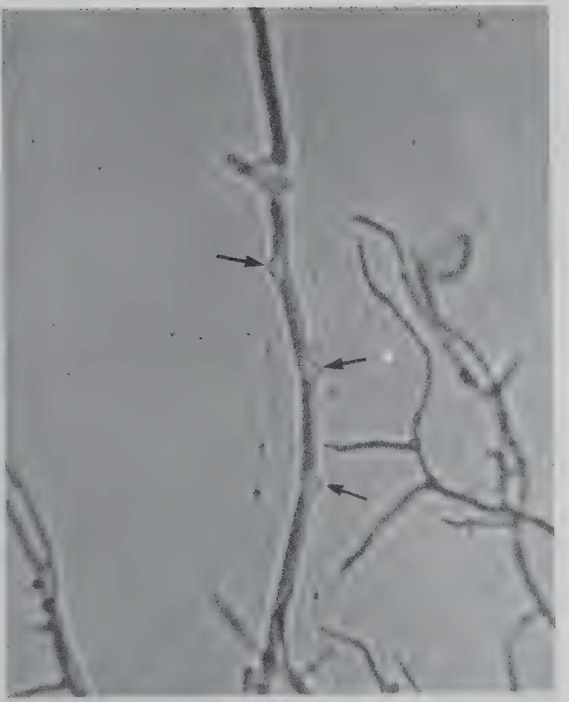

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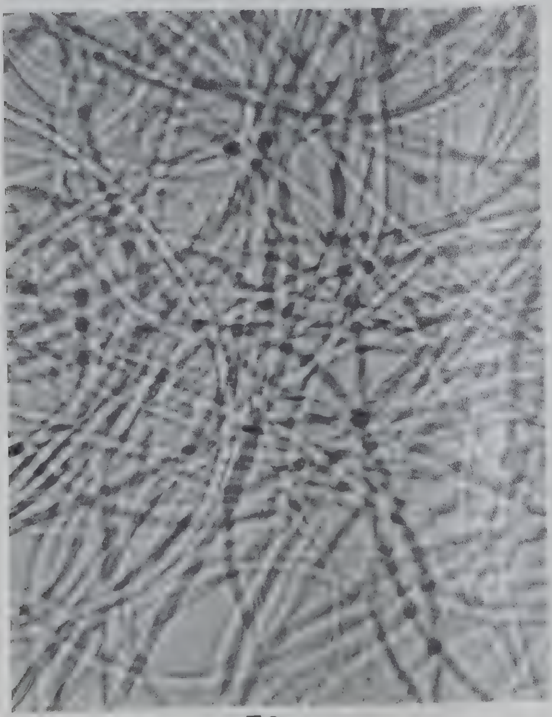

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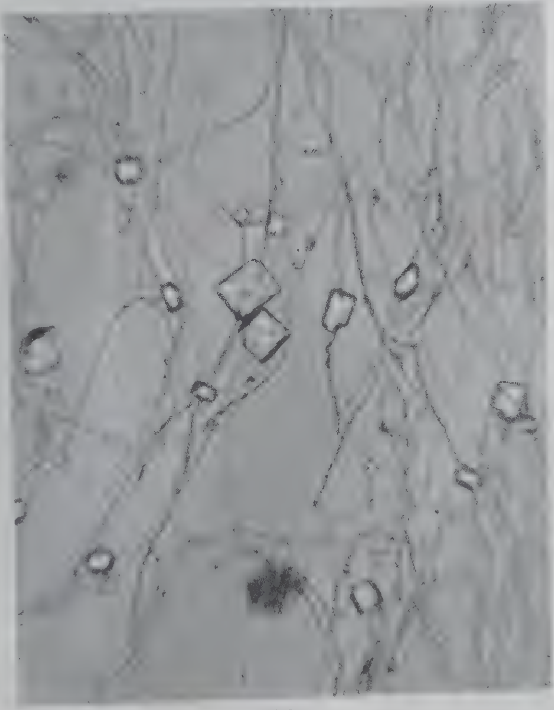

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Figure 72. Daedaloid pores forming along the plate edge. New pores are developing to the inside. X1.3.

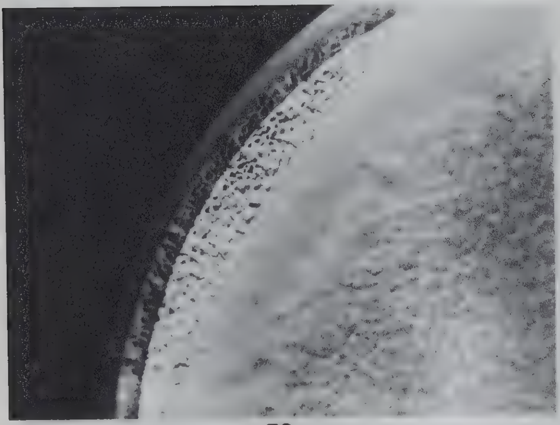
Figure 73. Pore walls developing on the compact, felty primordial area at the plate edge. The walls are irregular and incomplete at this stage. X5.6.

Figure 74. Rounded and sub-angular pore mouths of basidiocarp formed in agar culture. X5.6.

Figure 75. Daedaloid pore mouths of basidiocarp formed in agar culture. X5.6.

Figure 76. Apical ends of fiber hyphae protruding from the leading edge of the pore wall. X440.

Figure 77. Clavate, deeply stained basidia arising from the thin-walled, nodose-septate hyphae. Note the slender sterigmata. X440.



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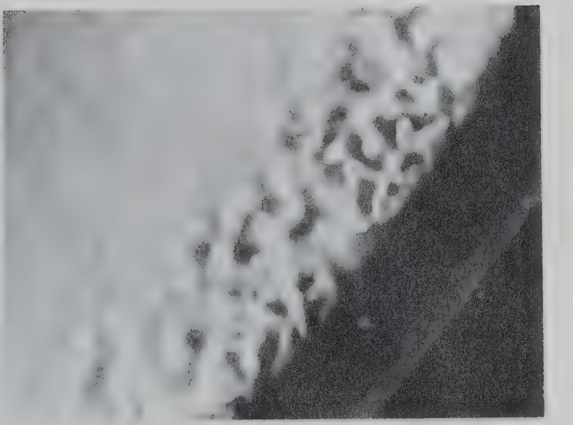

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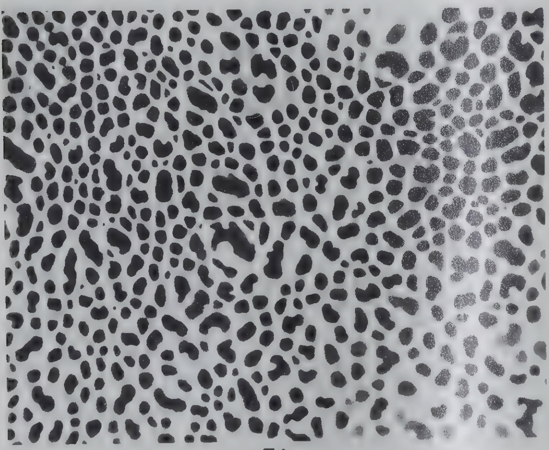

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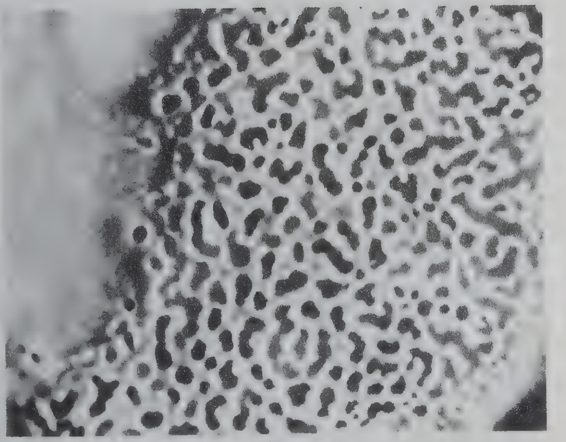

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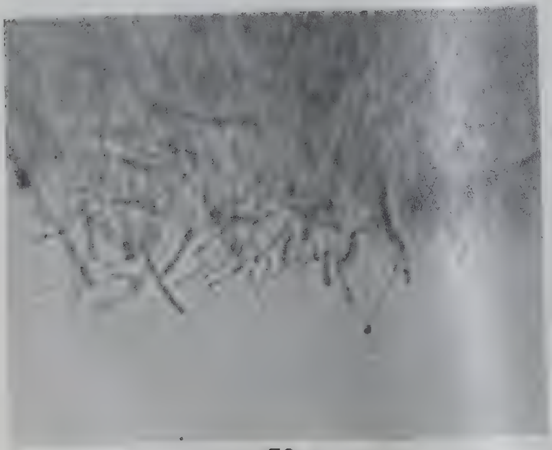

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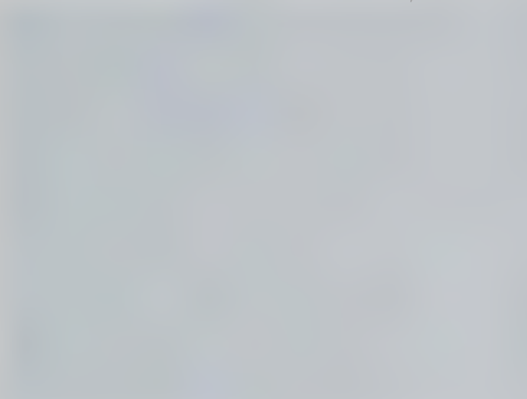
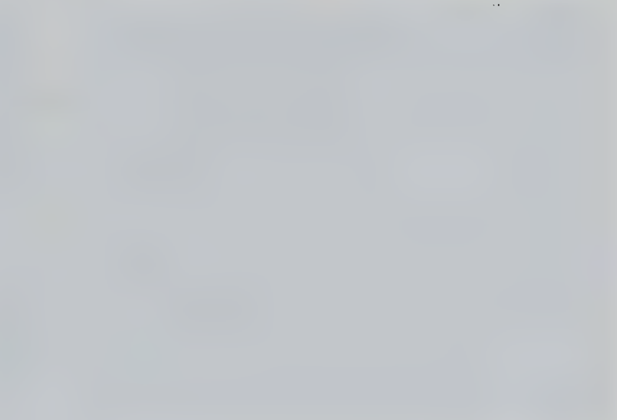
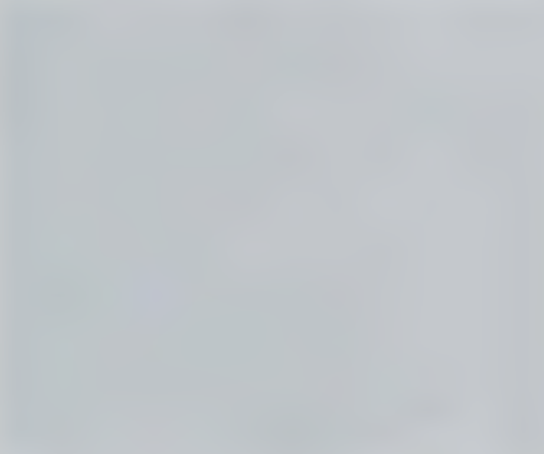
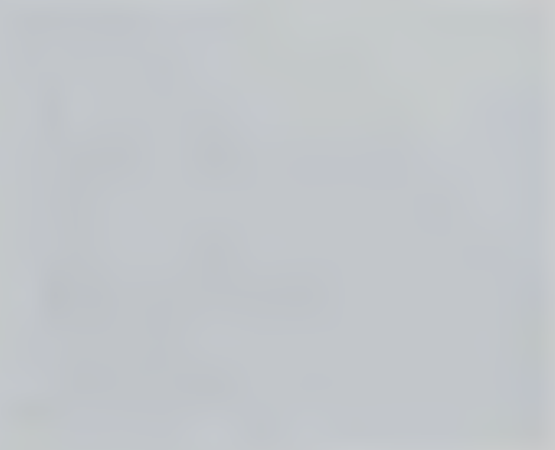








Figures 78-82. Still cultures of *F. cajanderi*, 2 months old, grown at room temperature. Note the various locations of primordial formation on the medium surface.

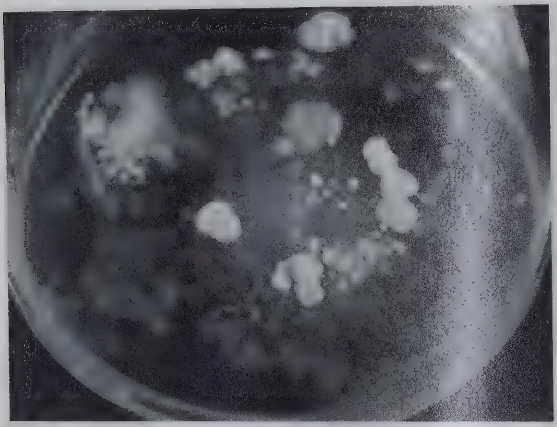
Figure 78. Small primordia developing among large, hemispherical primordia. B-189, X0.95.

Figure 79. Large primordium (8 mm across) at the margin of the aerial mat. B-189, X1.2.

Figure 80. Primordia forming at the margin of the aerial mat. B-3, X0.95.

Figure 81. Two small primordia developing on the medium surface away from the aerial mat. B-128, X1.2.

Figure 82. Numerous primordia forming in scattered areas of the aerial mat. B-189, X0.95.



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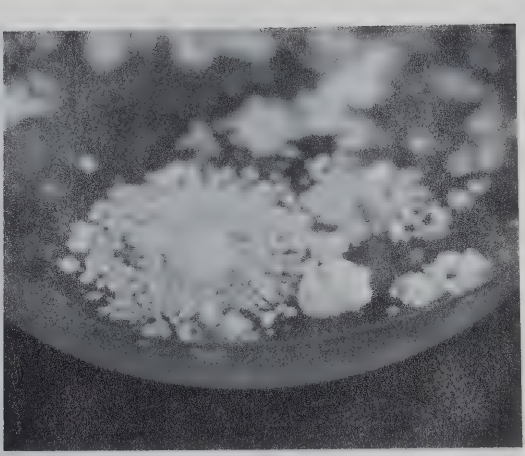

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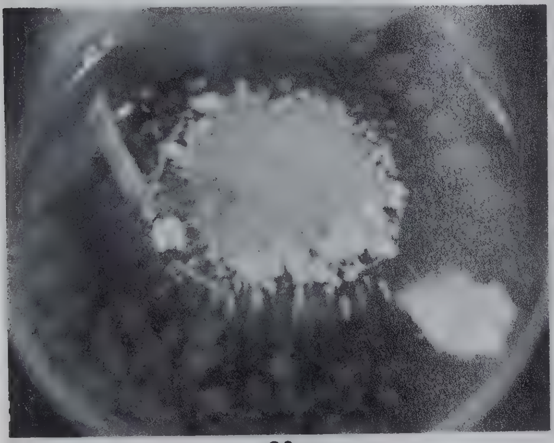

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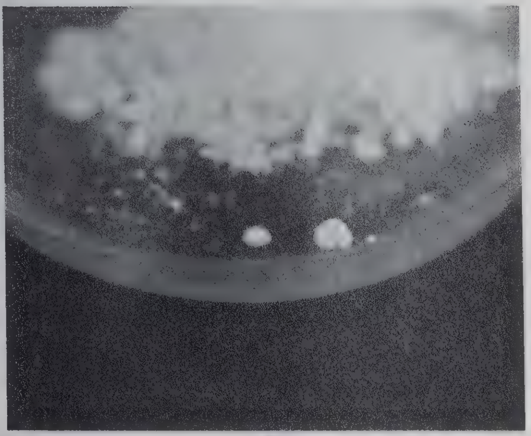

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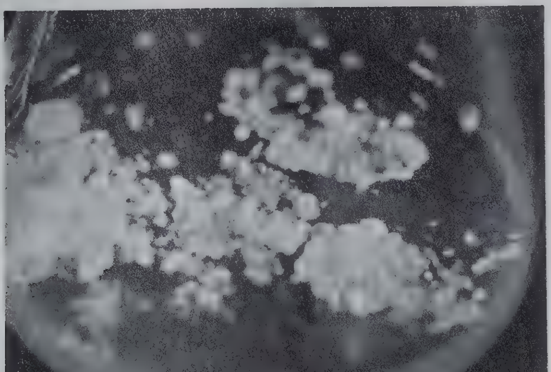

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Figure 83. Still culture of *F. cajanderi* (B-3), 4 months old, grown at room temperature. Note the extensive pore development on the aerial mat. X1.2.

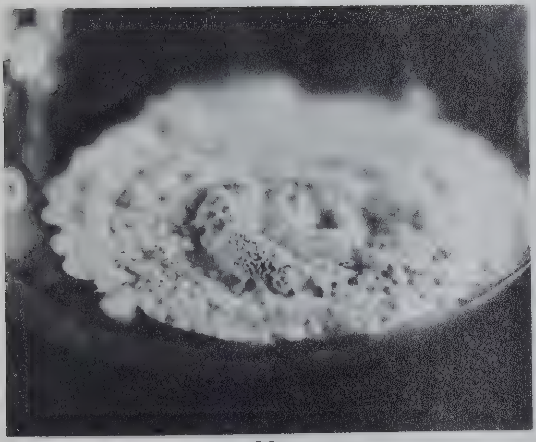
Figure 84. Still culture of *F. cajanderi* (B-3), 4 months old, grown at room temperature. The widely separated pores (arrows) give the mycelium a pitted appearance. X1.2.

Figure 85. Leading edge of pore wall consisting of lightly stained, apical portions of fiber hyphae. X440.

Figure 86. Apical portion of fiber hypha from the edge of the pore wall. The lumen is lightly stained and the walls are thin. X1125.

Figure 87. Densely intertangled and interwoven fiber hyphae of the interior of the pore wall. X440.

Figure 88. Deeply stained, clavate basidia from the pore wall of a basidiocarp formed in still culture. One basidium bears sterigmata and immature basidiospores. X1125.



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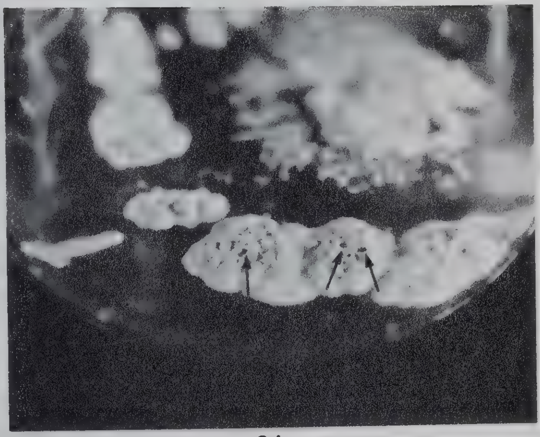

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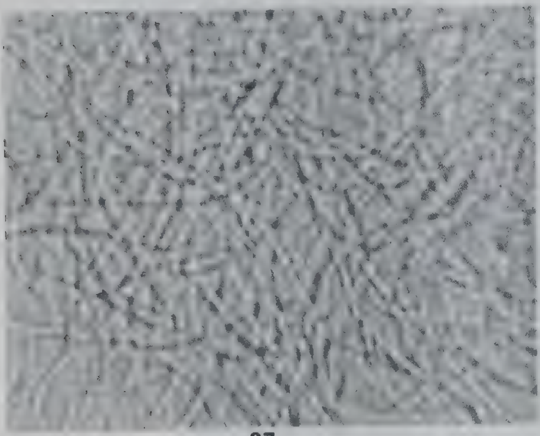

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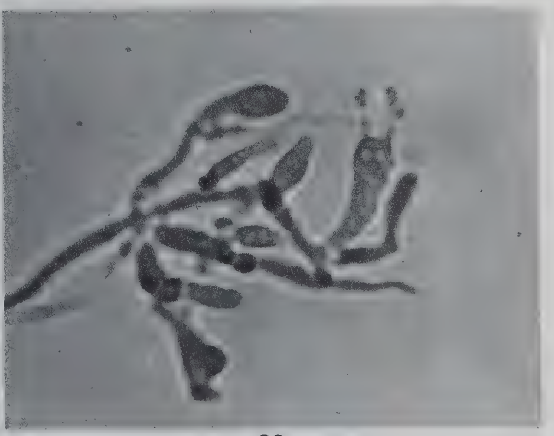

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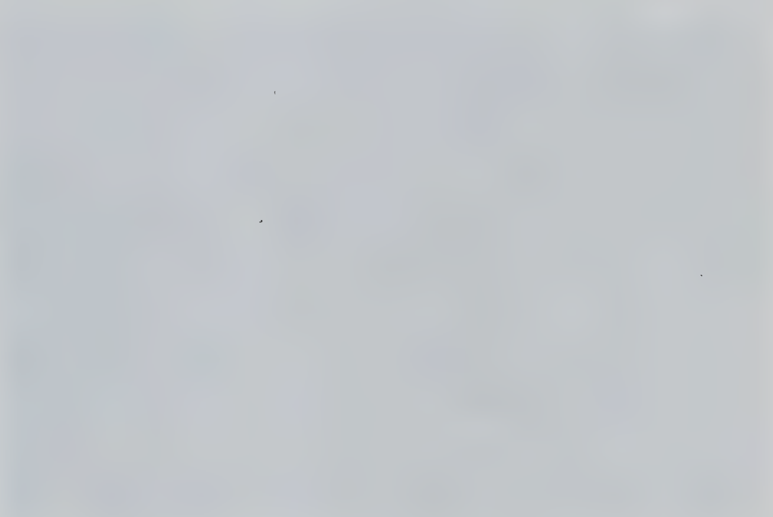
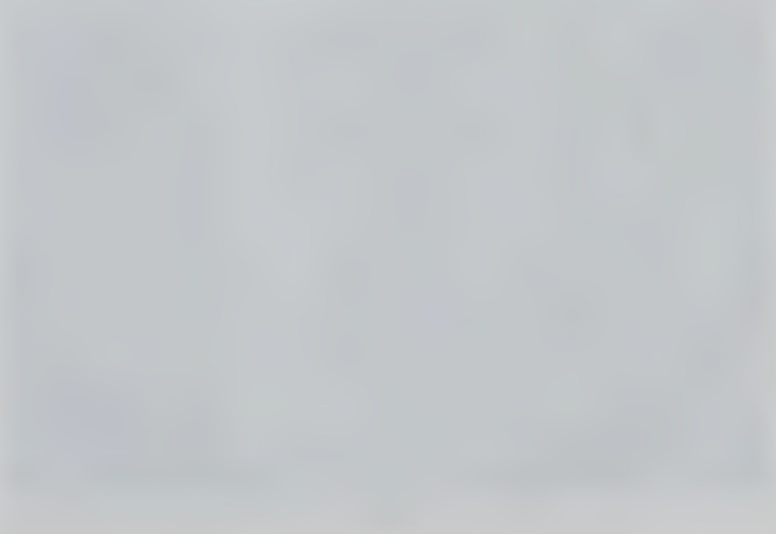


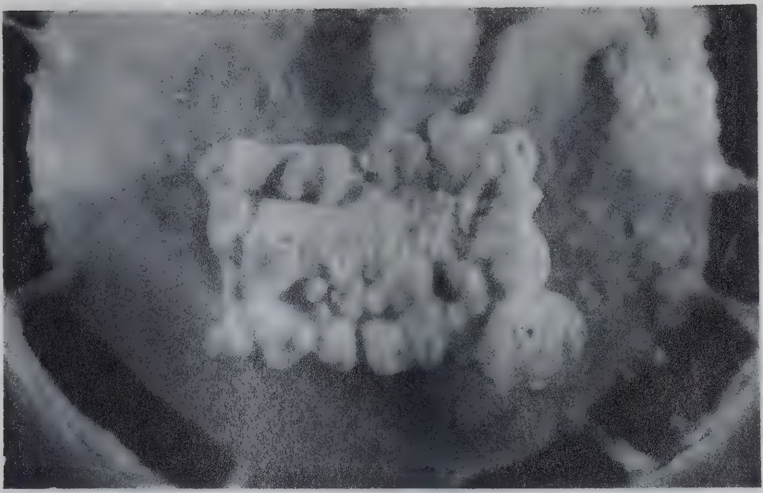


Figures 89-91. Compact, felty, hemispherical primordia developing along the top surface and top edges of wood blocks. All cultures are 12 months old.

Figure 89. Pine block culture of *F. cajanderi* (B-128) grown at room temperature. X1.3.

Figure 90. Pine block culture of *F. cajanderi* (B-189) grown at room temperature. X1.6.

Figure 91. Spruce block culture of *F. cajanderi* (B-3) grown at 20°C. Note the uneven, hummocky appearance of the mycelial mass on the block surface. X1.3.



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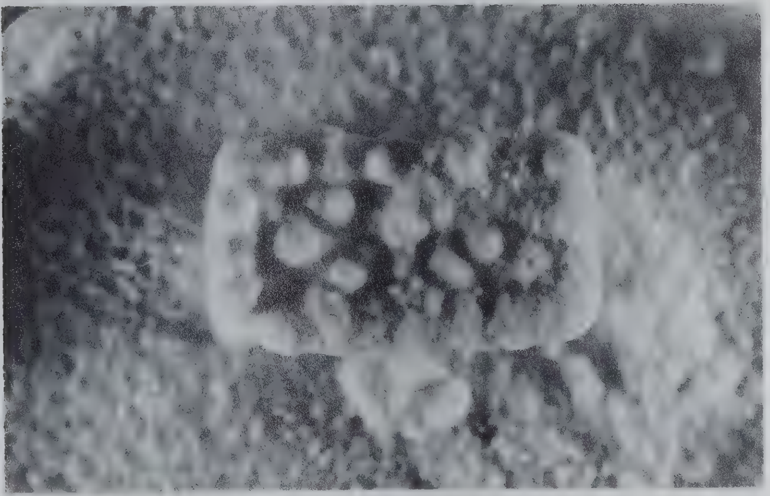

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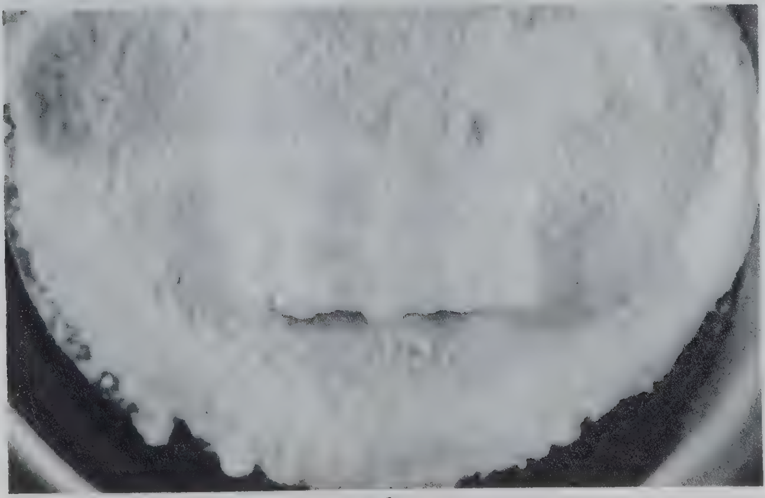

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Figure 92. Aspen poplar block culture of *F. cajanderi* (B-128) grown at 20°C for 10 months. Shallow furrows and ridges are developing on the primordial surface. X0.95.

Figure 93. Pine block culture of *F. cajanderi* (B-128) grown at room temperature for 12 months. Daedaloid pore mouths. X0.95.

Figure 94. Spruce block culture of *F. cajanderi* (B-128) grown at room temperature for 12 months. Daedaloid and rounded pore mouths. X1.3.

Figure 95. Aspen poplar block culture of *F. cajanderi* (B-128) grown at room temperature for 12 months. The widely separated, rounded pore mouths give the mycelial surface a pitted appearance. X0.95.

Figure 96. Spruce block culture of *F. cajanderi* (B-128) grown at 20°C for 10 months. Elongated, open pores have formed along the side of the wood block. X1.3.

Figure 97. Pine block culture of *F. cajanderi* (B-128) grown at room temperature for 12 months. Elongated, open pores have formed down the side of a primordium at the edge of the wood block. Note the incomplete pore walls (arrow). X1.5.

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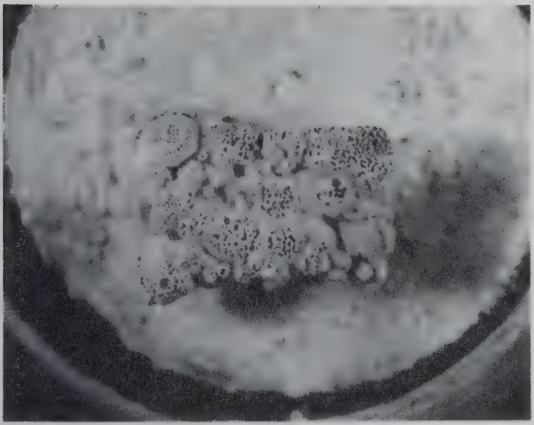
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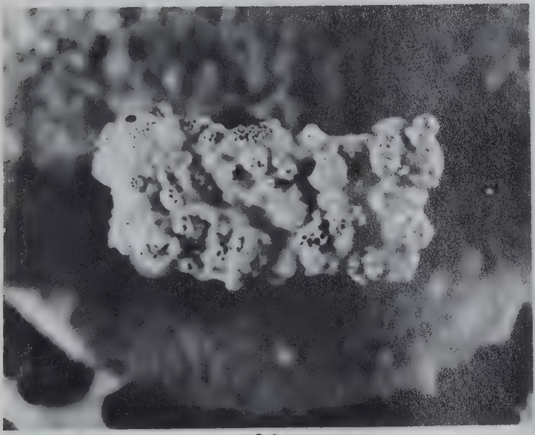
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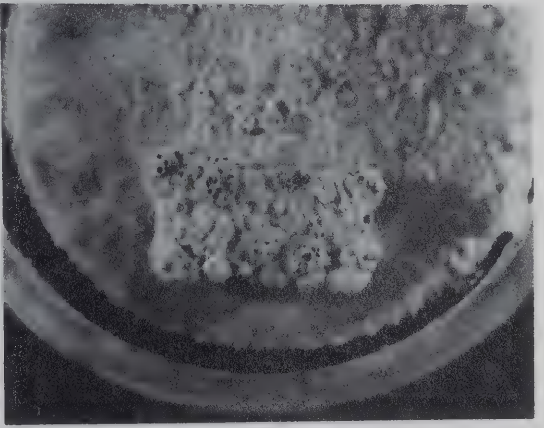
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Figure 98. Irregularly thickened, nodose-septate hypha from the primordium. The clamp connections have broken leaving the hook portions on the hypha. X1480.

Figure 99. Edge of a hemispherical primordium. The fiber hyphae are densely intertangled and the hyphal ends protrude outward from the edge. X180.

Figure 100. Edge of an elongate, open pore. The fiber hyphae protrude outward from the edge of the pore wall in a horizontal manner. X180.

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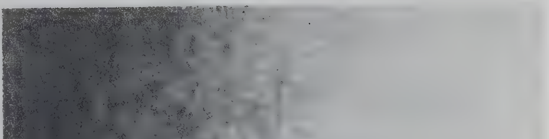

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DISCUSSION

The observations and results of this investigation have provided information on the development of *F. cajanderi* in nature and in culture. The growth of the vegetative mycelium and the formation of basidiocarps in both environments have been described more comprehensively than has been previously reported. In addition, exploratory work has been done on the effect of various carbon sources on growth and the effect of L-asparagine on the decay of wood-shavings. Temperature studies have confirmed earlier work that *F. cajanderi* grows at a moderate rate in agar culture. The following discussion will be presented in three major sections. The first will deal with the development of the vegetative mycelium and basidiocarps in nature. The second section will examine the results of growth of this species in culture. The third section will evaluate the taxonomic position of *F. cajanderi* using information of the basidiocarp structure and the characters of the cultural mycelium. The usefulness of the cultural mycelium in determining taxonomic relationships will be explored.

A. Development in nature

The vegetative mycelium in the naturally-infected and artificially-infected wood was similar in appearance in the early stages of decay. The mycelium is sparse and grows in a sinuous manner along the lumina of the tracheids, usually parallel to the long axis of the cells. In the naturally-infected wood examined only the dikaryotic mycelium has been observed. Zycha and Knopf (1966) have reported isolating monokaryotic colonies of several polypore species from decayed wood. However, Larcade (1970) found only the dikaryotic mycelium in wood infected by *Polyporus* (*Bjerkandera*) *adustus*. Also, Adams and Roth (1969) recovered numerous dikaryotic isolates of *F. cajanderi* from the heartwood of Douglas fir, but no mention is made of monokaryotic isolates being found. Formation of basidiocarp primordia in monokaryotic cultures of *F. cajanderi* has been reported by Neuhauser and Gilbertson (1971), but these structures were sterile. It appears, therefore, that the dikaryotic stage is predominant in the life cycle of *F. cajanderi* in naturally-infected wood. In the advanced stages of decay the mycelium is often found in dense aggregations in the tracheids near the ray cells. The hyphae, on rare occasions, bear "medallion" clamp connections. As noted earlier, the presence of "medallion" clamps on the vegetative hyphae of *F. cajanderi* has not been previously reported. States (1969) found that the frequency of "medallion" clamps decreases and normal clamps

are formed as the hyphae of *Gloeophyllum saepiarium* progress toward an opening in the wood. Due to the scarcity of "medallion" clamps on the hyphae of *F. cajanderi*, a correlation of the frequency of these clamps and the area of wood where they were observed could not be made. It was noted, however, that the hyphae emerging from the wood did not bear "medallion" clamps.

Proctor (1941) has suggested that fungi may be identified by their hyphal characteristics in decayed wood. This does not appear to be applicable to *F. cajanderi*. The vegetative hyphae of this species in decayed wood show little differentiation and very few distinct hyphal characters are present to allow one to identify the fungus. The hyphae are narrow, thin-walled, clamped, and hyaline, and they grow in a sinuous manner through the tracheids, often forming dense aggregations near the ray cells. The tracheids are penetrated via the bordered pits or by bore holes which are wider than the infecting hyphae. All these features are common to a number of polypore species. The occurrence of "medallion" clamps and cracked bore holes are of limited diagnostic value due to their irregular and sporadic appearance. Roff (1964) has stated "while it may be possible to differentiate between certain classes of wood-inhabiting fungi on the basis of their hyphal characteristics as seen in wood section, cultural tests are usually required to identify the species." In cases where basidiocarps of *F. cajanderi* are absent or at a very

immature stage, identification of the fungus in decayed wood must be based on a combination of characters such as macroscopic and microscopic appearance of the wood and cultural features. The value of the cultural mycelium in identification will be discussed later.

The mycelium near the wood surface emerges through cracks and fissures in the wood to form the primordium. Differentiation of the vegetative mycelium occurs at this stage in which some of the hyphae become thick-walled, brown-colored, and aseptate. The modification of the hyphae near the wood surface or emerging from the substrate is probably a response to the drastic change in environmental conditions that is encountered upon emergence. In the open the mycelium is subjected to greater light levels, higher oxygen concentrations, desiccation, and greater fluctuations in temperature. In addition, the mycelium is no longer in direct contact with the substrate so nutrients must be transported from the mycelium in the substrate to the hyphae forming the primordium.

An interesting aspect of wood infected by *F. cajanderi* is the frequent occurrence of red to reddish-brown stained areas. These areas have been variously described by other workers as "zone lines", "dark lines", "dark zones", or "discolored areas." The stained areas are due to dark-brown hyphal aggregations in the tracheids and a reddish-brown staining of the tracheid walls. In some instances, a dark-brown, amorphous substance is also seen among the

hyphae. The specific reason for the stained areas is not yet known, although cultural studies by Adams and Roth (1967, 1969) have provided strong evidence of its cause. These investigators have found that paired dikaryotic cultures of *F. cajanderi* form lines of demarcation at the interface or contact zones of the colonies. A discoloration caused by darkening of the mycelium and medium may occur in the contact zone depending on the genetic relationship of the cultures. Cultures distantly related formed demarcation lines 95-100% of the time, while cultures of closest relationship formed lines approximately 50% of the time (Adams and Roth, 1967). Further evidence was provided when cross-sections of Douglas fir infected by *F. cajanderi* were incubated for 5 months in sealed polyethylene bags. During this period surface mats of the fungus developed over the cross-sections, and demarcation lines formed between the various mats (Adams and Roth, 1969). In macroscopic appearance the lines were similar to those occurring in nature. Multiple infections were evidently occurring in the Douglas fir in which a number of colonies were contributing to the decay of the tree. The findings of these workers revealed an average number of 2.3 genotypes of *F. cajanderi* infecting 49 glaze-damaged Douglas fir trees.

It is possible that the stained areas in the wood of this study are due to interactions between different colonies of *F. cajanderi* competing for the substrate.

However, it must be noted that the Douglas fir trees examined by Adams and Roth were living and were almost exclusively infected by *F. cajanderi*. All wood samples in this study were obtained from stumps and logs and other species of wood-decay fungi were probably present in the wood. Thus secondary infection is a complicating factor which may be involved in formation of the stained areas. Further investigation is needed to explain their formation.

The macroscopic descriptions of the basidiocarps of *F. cajanderi* examined in this study agree in essentials with those of the literature. The basidiocarps are usually sessile or effused-reflexed in form, although the resupinate form has been noted in collections B-333. This specimen appears to be a rare exception since no mention is made of resupinate forms in the literature. The young, developing effused-reflexed basidiocarps may be temporarily resupinate and thus can be mistaken for a resupinate form. It is important, therefore, that the degree of development be taken into account when examining these young basidiocarps, as well as their position on the substrate. The resupinate form would develop in situations unfavorable for the normal growth form of the basidiocarp, such as on the underside or lower portions of large logs. Since *F. cajanderi* basidiocarps are often found in small groups, examination of the other basidiocarps present would probably show some attempt at pileus formation, indicating that the fungus is not normally a resupinate species.

The pileus form is applanate or convex in fully developed specimens, but young specimens can be somewhat ungulate. The ungulate form has led to confusion with *F. roseus* which is often ungulate. However, the darker-tinted context and the lack of a crust are two features of *F. cajanderi* that distinguish it from *F. roseus*. The context of *F. roseus* is silvery-pink, while the context of *F. cajanderi* is rosy-pink to brown.

The basidiocarps that develop in the large, lateral cracks of logs may become laterally fused and confluent to form long bands. In this study, fused basidiocarps have measured over 20 cm, but Shope (1931) has reported basidiocarps over 50 cm in length. Due to the tendency of basidiocarps to fuse laterally when developing in horizontal cracks, the measurements of maximum lengths for the species may be somewhat variable. Because of this variability, measurements given in this thesis are the maximum lengths of single basidiocarps that have not fused. The lengths of laterally fused basidiocarps are presented separately as is the case in most descriptions of the species in the literature. It should be noted that basidiocarps of *F. roseus* do not show the tendency to fuse laterally into long bands, but are usually found as solitary basidiocarps, or at most, only touching each other or partly fused.

The basidiocarps of *F. cajanderi* examined during this study were all found growing saprophytically, usually on conifer logs and stumps. The saprophytic nature of the

fungus appears to be the general rule since references reporting the occurrence of *F. cajanderi* on living trees are rare. Zeller (1926) noted that extensive damage to living peach and prune trees was due to this polypore, which was described as a wound parasite occurring on pruning cuts and broken branches. Damage was not limited to the heartwood, but could spread to the sapwood causing injury to the rest of the tree. Snell, Hutchinson, and Newton (1928) used a culture of *F. cajanderi* (as *Trametes subrosea*) isolated from the exposed heartwood of a living cherry tree in their temperature studies. As noted before, Adams and Roth (1967, 1969) isolated numerous colonies of *F. cajanderi* from living, glaze-damaged Douglas firs. In all these examples, the fungus had attacked initially the wounded parts of the tree, notably the heartwood. The ability to attack the wounded portions of a living tree does not imply that the fungus can attack the living cells. To provide evidence of this, living, healthy trees would have to be artificially-infected, and the living tissues examined at a later date to show that the hyphae are attacking and causing the death of the cells. Unfortunately, no work of this kind has been done with *F. cajanderi*. Other workers have used different polypore species such as *Fomes* (*Ganoderma*) *applanatus* (White, 1919) and *Polyporus* (*Piptiporus*) *betulinus* (Henningsson, 1965) and successfully infected living trees with these fungi.

The hyphal analysis of *F. cajanderi* has revealed that

this polypore is dimitic with generative and skeletal hyphae present in all parts of the basidiocarp. The skeletal hyphae observed in this species, however, differ slightly from Corner's original concept of skeletal hyphae as first observed in *Polystictus xanthopus* (Corner, 1932a). The skeletal hyphae in *F. cajanderi* sometimes are branched, with branches arising from the apical or central portions of the hyphae. This type of skeletal hyphae has been named arboriform by Teixeira (1962) because of their tree-like form. In the basidiocarps of *F. cajanderi* the branched portions of the skeletal hyphae appear to have a binding function, since they are interwoven among the hyphae to create a firm, compact mass. The straighter main portions of the hyphae and the branched portions may be an intermediary type of structure with a skeletal and binding function. Edwards (1972), when discussing the microstructure of *Coriolus hirsutus* and *C. pubescens*, points out that the skeletal elements (hyphae) in these two species are branched at their distal ends with the branches growing between and interweaving among other skeletal elements. As the branches tie the context hyphae together to some extent, he believes they act as binding hyphae. It should be pointed out, however, that these branched skeletal hyphae are not binding hyphae in the original sense of Corner. The binding hyphae as defined by Corner (1953) are much-branched, thick-walled hyphae of very intricate and limited growth, developing behind the growing point

(margin). In the species examined, the branched skeletal hyphae develop in the marginal region and they do not show intricate and limited growth. Hansen (1958) describes the skeletal hyphae of several *Ganoderma* species that she studied as belonging to a skeletal-binding system. She states "though the stems are always longitudinal and act as a framework, the tapering branches function as ties." The branched skeletal hyphae that she observed were of the arboriform type. Hyphal analysis of *Fomes levigatus* by Corner (1932b) revealed that this polypore was dimitic, with generative and skeletal hyphae being present. In this fungus the skeletal hyphae are unbranched, or at most, bear only short projections or spines. Their role in the microstructure of the basidiocarp is defined as a framework, with no transition between a skeletal and binding function.

The skeletal hyphae in the basidiocarp of *F. cajanderi* develop in the marginal regions. This is in agreement with Corner who noted that the skeletal hyphae always arise in the growing margin. A skeletal hypha arises as a terminal cell of a thin-walled generative hypha to which it is attached by a clamp connection. As the developing skeletal hypha increases in length, wall thickening in the central portions occurs. In *F. cajanderi* basidiocarps, wall thickening begins when the developing skeletal hypha is approximately 100 μ in length. Corner (1932b) found that wall thickening in skeletal hyphae of *F. levigatus* began when the hyphae were 100-300 μ in length, while Edwards

(1972) noted that the developing skeletal hyphae were thin-walled until they were 200-500 μ in length. Edwards also pointed out that the growing margin was composed of elongating apices of end cells which develop into skeletal elements. Generative hyphae were not found in the marginal region. In *F. cajanderi* basidiocarps developing skeletal hyphae and generative hyphae were both observed in the marginal region. In *F. levigatus* basidiocarps, the margin consisted of radiating ends of skeletal and generative hyphae. I believe this difference in observations may be due to each investigator's interpretation of the marginal region. In this thesis the marginal region is considered to be the area between the outer edge of the basidiocarp and 2000-3000 μ back from the edge. This area is considered marginal since it is at the edge of the basidiocarp where skeletal hyphal formation and elongation are occurring. Corner and Edwards do not mention the distance back from the outer edge of the basidiocarp that they consider marginal. Thus it is possible that Corner's idea of the margin may extend further back into the basidiocarp than Edwards' interpretation of the margin. The marginal region in my concept and in Corner's concept may be submarginal by Edwards' definition. In the submarginal region Edwards notes that generative hyphae are found. The important aspect of the marginal region, however, is that skeletal hyphal development occurs here and is the result of elongating, terminal cells of the generative hyphae. In effect, the marginal region is

actually the meristematic region.

The skeletal hyphae in the context of *F. cajanderi* basidiocarps are fully developed with thick, brownish walls and narrow, lightly stained lumina. The hyphae are arranged in a longitudinal direction. The branches of the skeletal hyphae are also fully developed and are interwoven among the longitudinal hyphae. The most interesting feature of the context region is the presence of differentiated generative hyphae. These hyphae have developed irregularly thickened walls and are contorted and tortuous in form. They represent a substantial part of the context and are usually seen in dense masses or "islands" among the skeletal hyphae. In a number of polypore species the generative hyphae disappear completely in the context region, and are only observed in the marginal regions. Teixeira (1960) describes some polypores where this is prevalent. He gives *Fomes fomentarius* and *Ganoderma applanatum* as two examples where the generative hyphae collapse completely. In a number of other polypore species differentiation of the generative hyphae does occur, usually in the form of wall thickening. The species include *Coriolus hirsutus* and *C. pubescens* (Edwards, 1972), *Cerrena unicolor* (van der Westhuizen, 1963), *Polystictus microcyclus* (Corner, 1953), and *Fomes levigatus* (Corner, 1932b). In regards to *F. levigatus*, Corner states "many of the generative hyphae at the base of the dissepiments and in the flesh immediately above have become thick-walled and rather

intricately interwoven, even irregularly lobed, and in thus assuming to a small extent the appearance and function of the binding hyphae of *P. xanthopus* they help to strengthen the layer of flesh above the tubes." The irregularly thickened generative hyphae in the context region of basidiocarps of *F. cajanderi* may contribute to the rigidity of the structures by acting as ties between the skeletal hyphae.

Stable taxonomic criteria are essential in setting up a classification, and when macroscopic features that are variable are chosen artificial systems are often the result. The highly variable appearance of the pileus surface of *F. cajanderi* basidiocarps is indicative of some of the shortcomings of macroscopic descriptions of this species. Depending on the stage of development and the effects of weathering, the pileus surface may be finely tomentose to radiately fibrillose, glabrous to furrowed, zonate or azonate, and light-pink to grayish-white. The surface features are also reflected in the appearance and arrangement of the hyphae that compose the surface. In the tomentose surface the generative and skeletal hyphae are in small tufts, while in the older areas the skeletal hyphae predominate and are agglutinated to form a firm, compact mass. In the weathered parts of the pileus surface, the skeletal hyphae are bleached and colorless in the upper 30-100 μ portions. The ontogenetical details of the surface layers have not been examined in most polypore species. The observations of the hyphae that compose the pileus

surface of *F. cajanderi* basidiocarps are descriptive and do not give precise details of the formation of the surface. Further investigation is required to determine how the surface tissues arise from the generative and skeletal hyphae. Only a small aspect of the pileus surface, the zonation, will be discussed here.

Since the basidiocarps of polypores grow by the accretion of marginal hyphae over a period of months or years, the marginal region is exposed to many different atmospheric and substrate changes. These changes may be shown in the surface coloration. Austwick (1968) has pointed out that the zonation of the pileus surface of the bracket fungi can give much information on the suitability of the weather for rapid or for slow growth during the period of development. He notes that generally, under conditions of high humidity and mild temperatures growth occurs rapidly, but under dry, frosty, or excessively wet conditions, growth may cease abruptly. The rate of growth will influence the colored zones, since slowing up of hyphal elongation tends to accumulate protoplasm in the hyphal tips, where pigment may also concentrate (Austwick, 1968). The influence of changes in the atmosphere surrounding basidiocarps of *F. cajanderi* appear to be represented by the zonation of the pileus surface. In the zones the hyphae have dark walls and dark reddish-brown contents which may be the result of unfavorable growth conditions. The exact relationship of environmental

factors to zonal types requires more investigation.

Irregular zonation in the context is equally difficult to account for in terms of environmental influence. With very few exceptions, e.g., Larcade, 1970, almost no observations of humidity, rainfall, temperature, light, etc., have been taken during the development of the basidiocarps. Observations of *F. cajanderi* basidiocarps reveal that at the edges of the zones there are deeply colored skeletal hyphae with dark contents. It appears that during unfavorable conditions, such ~~as~~ desiccation, most of the hyphal tips have become agglutinated and die. When favorable growth conditions return, new growth is started by lateral branches behind the zone edge and hyphae grow between the dead hyphal tips. Occasionally, new growth begins from the edge of the pore field and extends up and over the old surface to form a new margin. The skeletal hyphal tips may show renewed growth in which the result is a constriction in the hyphae. Edwards (1972) observed similar constrictions in the hyphae of *C. pubescens*. He thought the temporary cessation of growth of the apices allowed wall thickening to catch up to the apices resulting in slight thickening of the walls. When growth resumed only the very tips of the hyphae elongated so a constriction is formed.

The "white, stuffed" appearance of the older pores in basidiocarps of *F. cajanderi* is caused by thin-walled generative hyphae growing into the pore space. The hyphae appear to develop from the generative hyphae of the

dissepiments and from sterile basidia lining the pore wall. Corner (1932b) noted that in *F. levigatus* the older pores were also filled with hyphae which he called "stuffing hyphae." These hyphae were narrow, aseptate, and thickened with pale yellowish walls. They developed from the generative hyphae of the dissepiments and from the vegetative mycelium growing into the context from the base of the basidiocarps. In the pores of *F. cajanderi* basidiocarps, no hyphae could be traced from the mycelium at the base of the basidiocarp growing into the pore space. The development of elongated hyphal tips from basidia has been reported by States (1969) in basidiocarps of *Gloeophyllum saepiarium* that were stored in a cool, moist container . It is possible that in the natural environment the basidia in pores of *F. cajanderi* basidiocarps respond in a similar manner under cool conditions.

In concluding this section, it can be reported that the macrostructure of *F. cajanderi* is highly variable due to the effects of weathering and the stage and position of development. There may be an overlap in certain morphological features of basidiocarps of this species and *F. roseus* which can lead to confusion between these two similarly-colored polypores. The microstructure of *F. cajanderi* basidiocarps has revealed that they are dimitic with generative and skeletal hyphae. The generative hyphae show distinct differentiation in that the walls become irregularly thickened. Also the clamp connections become

swollen and solid and are easily broken. Solid, short protuberances may develop along the thickened walls. The skeletal hyphae are somewhat distinct in that they occasionally develop branches in their apical and central portions. These branches appear to have a binding function while the longitudinal portions of the main hyphae act as a framework.

B. Development in culture

1. Growth of the vegetative mycelium

The vegetative mycelium of *F. cajanderi* in the three types of cultures showed a remarkable uniformity in hyphal types. In all three types of cultures thin-walled, nodose-septate hyphae, irregularly thickened, nodose-septate hyphae, and fiber hyphae were observed. The relative amounts of each hyphal type varied in each of the cultures, but the mode of development of the irregularly thickened, nodose-septate hyphae and the fiber hyphae was identical in all cultures. Slight variations did occur in the hyphal diameters and in the number of branches that developed on a fiber hypha, but overall there was uniformity of form and development of each of the hyphal types in the three cultures.

The irregularly thickened hyphae developed from the thin-walled, nodose-septate hyphae. The first indication of wall thickening was the development of swollen, solid

appearing clamp connections and short, solid protuberances along the hyphal walls. The walls later became irregularly thickened, refractive, and hyaline. The lumen was observed as a narrow, uneven line passing from side to side in the hypha. The fiber hyphae developed from the terminal cells of the thin-walled, nodose-septate hyphae. A fiber hypha initially grows as a long, flexuous, hyaline, aseptate cell, its diameter being the same as the hypha from which it arises. As the developing fiber hypha grows, its diameter gradually increases until it may be 2-3 times the diameter of the proximal end. Wall thickening occurs in the developing fiber hypha when it is 50-100 μ in length. The central portions of the fiber hypha thicken first and this wall thickening process continues towards each end of the hypha. The lumen becomes narrower and the contents appear faintly stained as wall thickening continues. The thickened walls appear refractive, colorless, and may reach 1 μ in thickness. Fully developed fiber hyphae may measure over 1500 μ in length.

The descriptions of the agar cultures given in this study agree reasonably well with the descriptions of *F. cajanderi* by Nobles (1965). She reported that agar cultures of *F. cajanderi* are pink or vinaceous, cottony to cottony-floccose, pitted, chlamydospores rare, 10.5-24.0 x 6.0-7.5 μ, thin-walled, fiber hyphae occasionally branched, hyphae with walls irregularly thickened. Tests for extracellular oxidase were sometimes erratic, i.e., at times a color change

occurred, at other times no color change occurred. I have found that the central parts of the plates were cottony to cottony-floccose, but the edges often became dense, compact, and felty. Pitted areas were not observed in the cultures. The range of chlamydospore sizes given was similar to the findings of this study. In all tests for extracellular oxidase, using an alcoholic solution of guaiacum, no positive test resulted. Gallic or tannic acid agar plates were not used in the tests. It is possible that, if these had been used, a positive reaction for extracellular oxidase would have occurred. According to Nobles (1971), the color changes that occur are probably caused by one or more laccases released by the cells into the medium. Irregularly thickened hyphae and branched fiber hyphae were observed in the cultures in agreement with Nobles' descriptions. Fiber hyphae had developed as described by Nobles, as elongated terminal cells of hyphae.

The hyphal types found in the mycelium of cultures are similar to those found in nature. Edwards (1972) noted in *Coriolus* species that little superficial growth occurred over the wood except in places where humidity was high such as beneath logs or in cracks in the wood. In this study was noted that light-pink to white mycelial mats occasionally formed in deep cracks of wood under moist, shaded conditions. This mycelium is like the aerial mycelium grown in culture and examination of these mycelial mats showed that two hyphal types were present.

One was long, flexuous, thick-walled, aseptate, refractive, and 0.9-3.5 μ in diameter. These hyphae were sparingly branched and loosely intertangled to form a cottony to cottony-floccose mat on the wood surface. The second hyphal type, which was intertangled among the thick-walled hyphae in an unorganized manner, was thin-walled, hyaline, clamped, and 0.9-2.6 μ in diameter. The light-pink color appeared to be due to the light yellowish-brown walls of the thick-walled hyphae. These two hyphal types were identical in appearance to the fiber hyphae and thin-walled, nodose-septate hyphae produced in culture.

2. Physiological studies

The growth rate of 3 isolates of *F. cajanderi* on malt agar was maximal at 28°C with inhibition approached at 37°C. Snell, Hutchinson, and Newton (1928), using at least 5 isolates of the species, observed that maximal growth occurred at 30°C and was inhibited at 38°C. Humphrey and Siggers (1933), using only one isolate, reported optimal growth at 28°C and inhibition at 38°C. These results agree reasonably well with each other considering that slightly different media and methods of measuring radial growth were used in the studies. The two cardinal temperatures found for *F. cajanderi* support the contention of Cartwright and Findlay (1958) that cardinal points do appear to be fairly constant in any species. These workers also point out that even though the cardinal points are constant, there may be differences in the absolute rates of growth of different

isolates from separate areas. This was noted in this study and in the results of Snell, Hutchinson, and Newton. The optimal growth of *F. roseus* is reported by Snell, Hutchinson, and Newton (1928) to occur at 26-28°C. The growth rate of this polypore is decidedly slower than *F. cajanderi* at 30-32°C. These workers report that these two related species can be differentiated with absolute reliability by growing them at 30-32°C.

The results of the growth-curve experiment using dextrose-salts medium are similar to those obtained by other workers. Ward and Colotelo (1960), using a "low-temperature" basidiomycete growing in liquid synthetic medium, observed a lag phase during the first few days, followed by a rapid increase in growth, and finally, an autolysis phase where dry weight either decreased or where growth was counterbalanced by autolysis. The accumulation of toxic metabolites or shortage of nutrients may cause cessation of growth during the latter stages. With *F. cajanderi* growing in the synthetic medium, maximal growth was approached about 21-28 days after inoculation. Thus an incubation period of 23 days was chosen for the carbon utilization studies.

In the malt extract medium the growth of *F. cajanderi* showed no detectable lag phase. This was expected since the inoculum was transferred from a malt extract medium of the same composition. There would be no need for enzymatic adaptation by the fungus. The greater amount of growth in

the malt extract medium compared to dextrose-salts medium was also expected, since the malt extract probably contains greater amounts of carbon and/or nitrogen as well as unknown growth-factors and vitamins. Other workers have also reported greater growth on malt extract medium compared to most synthetic media (Jennison, Newcomb, and Henderson, 1955).

In both growth-curve experiments the pH dropped during the incubation period. In the dextrose-salts medium, the initial pH was 4.7-5.0 and after 36 days it had dropped to about 3.4. The malt extract medium showed a decrease in pH from 5.3 to 3.1 after 35 days. A decrease in pH was found by Henningsson (1965) using *Polyporus* (*Piptiporus*) *betulinus* and *Polyporus marginatus* working with a number of carbon sources. The pH changes may be due to production of acid substances by the fungus during the incubation period.

Good growth of *F. cajanderi* occurred with dextrose, D-mannose, and cellobiose and slightly less growth occurred with maltose. Little growth occurred with D-xylose, D-fructose, galactose, and sucrose. The negligible growth occurring in the negative controls shows that nutrient carry-over by the inoculum was minimal. Henningsson (1965) reported that the brown-rot fungi *P. betulinus* and *P. marginatus* both showed good growth with glucose, mannose, and cellobiose. He also pointed out that it is not unusual for cellobiose to give better growth than glucose. He suggests that certain impurities in cellobiose may account

for this growth effect. The small amount of growth with D-fructose was unexpected since this sugar and dextrose and mannose are closely related hexoses. It is possible that fructose is altered more by autoclaving than the other two which may affect its utilization by *F. cajanderi*. Comparisons of growth using autoclaved and filter-sterilized fructose should be done to substantiate this theory. A small amount of growth also occurred with D-xylose, galactose, and sucrose. Lilly and Barnett (1953) found that many of the 57 fungi they examined grew well on xylose and sucrose. They also reported that some of the fungi grew slowly on galactose at first but, after a time, depending on the experimental conditions and species, galactose was used readily. It is possible that the incubation period used in this study may not have allowed the fungus to adapt to the substrate on which it was growing. However, galactose supported only fair growth of *P. betulinus* with an incubation period of 35 days (Henningsson, 1965).

The results of the carbon utilization studies are difficult to compare with those reported in the literature because different experimental methods and different fungi were used. Also different isolates of the same fungus would probably yield slightly different results. Lilly and Barnett (1953) remark that fungi rarely, if ever, come in contact with a single carbohydrate in nature. They suggest that some carbohydrates which are not utilized by fungi when they are the only carbon source in a medium, may be

readily utilized in the presence of a utilizable simple sugar. Both *P. betulinus* and *P. marginatus* were able to decompose filter paper to only a small extent as reported by Henningsson (1965). However, under natural circumstances cellulose is utilized rapidly by these two brown-rot fungi. Henningsson postulates that in the wood substrate the presence of other carbohydrates may induce greater production of cellulases. The presence of other organisms breaking down cellulose into cellobiose may also be a factor that allows utilization of cellulose in nature. The effect of mixtures of carbohydrates on the growth of fungi should be investigated.

The results of the wood-shavings experiment revealed that both isolates of *F. cajanderi* decayed spruce shavings to a much lesser degree compared to the negative controls when relatively high concentrations of L-asparagine were added. At concentrations of 0.5% and greater, decay was substantially less than the controls and the cultures with 0.1% L-asparagine concentration. These findings were completely unexpected in that other workers had reported much greater weight losses when an organic nitrogen source was added to the wood. Findlay (1934) observed that the addition of asparagine to blocks of Sitka spruce caused a large increase in weight loss by *Trametes serialis*. Blocks treated with 1.0% asparagine lost 37.0% in weight while the controls lost 25.8% in weight. Schmitz and Kaufert (1936) reported that the addition of asparagine at 0.5% and 1.0%

concentrations caused significant increases in weight losses in sawdust cultures of *Lenzites trabea*.

It has also been found that addition of nitrogen and carbon sources can decrease the amount of wood decay. Schmitz and Kaufert (1938) investigated the effect of additions of dextrose or dextrose and asparagine to sawdust cultures of *Lentinus lepideus*. A significant decrease in weight loss occurred when these nutrients were added. These workers suggested that the fungus was utilizing the more readily available nutrients to produce large amounts of mycelium, but the sawdust was not been attacked as rapidly in the presence of nutrients as it was when they were absent. The fungus was developing largely at the expense of the dextrose and asparagine present and only to a limited degree at the expense of the wood substance. This does not appear to be the case in this study. Stimulation of mycelial growth of *F. cajanderi* occurred in the cultures containing little or no added L-asparagine. In the cultures containing L-asparagine at concentrations higher than 0.5% almost no mycelial growth occurred and only slight weight losses were recorded. Growth inhibiting substances may have formed when the wood-shavings and L-asparagine were autoclaved together. It should be noted, however, that Schmitz and Kaufert autoclaved their sawdust cultures together with the asparagine and observed no inhibition of growth.

3. Development of the basidiocarp

Fertile basidiocarps formed in all three types of

culture. Fruiting occurred readily in agar culture, less readily in wood-block culture, and very erratically in still culture. Normal appearing basidiocarps did not form in any of the cultures. The closest appearance to natural basidiocarps occurred in the agar cultures where the rounded to sub-angular pore mouths were similar to those found in natural basidiocarps. In the other types of cultures the pore mouths were often daedaloid and somewhat irregular.

An interesting aspect of the basidiocarp development was the various times at which primordial and pore formation took place. Fruiting occurred in 3-4 weeks in agar culture while in still culture and wood-block culture fruiting did not occur until 3-4 months after inoculation. It is possible that a shortage of nutrients may induce basidiocarp development in agar culture. The mycelium, upon reaching the edges of the Petri plates, no longer is able to obtain nutrients and its response is fruiting. Other factors may be involved such as aeration. Basidiocarp development frequently took place near the edges of the Petri plate where there may be better gaseous exchange from under the lid of the plate. The failure of *F. cajanderi* to fruit readily in still culture was unexpected since numerous primordia developed, but very few pores. Hawker (1966) points out that the frequent failure of fungi to sporulate in large volumes of liquid media may be attributed to the accumulation of carbon dioxide as a result of respiration. Factors such as the accumulation of

free ammonia or other volatile metabolic products may also be important. The slow development of pores in the wood-block cultures is somewhat similar to the findings of Mounce (1929) who was working with *Fomes pinicola*. She reported that the mycelium grew out from the inoculum and gradually covered the block. One year after inoculation primordia began to form.

The formation of pores in the three types of cultures was preceded by primordial development. The primordium was a compact, felty, light-pink to light-brown, raised mycelial mass. In all cultures the pores usually formed only on a small area of the primordium. Exceptions to this were noted in the wood-block cultures where pores occasionally covered the entire primordial surface. Pore formation was similar in all cultures. On the primordial surface shallow furrows and ridges formed. The upward or downward growth of the ridges, depending on the type of culture, resulted in formation of pore mouths. The pore mouths may be daedaloid, sub-angular, or rounded because of the different thicknesses and configurations of the pore walls.

The hyphal types observed in the basidiocarps were similar. The fiber hyphae, which composed most of the pore walls, grew in a downward or upward direction, depending on the type of culture. The hyphae interwove among each other to form a very coherent, dense pore wall. At the edges of the pore walls the apical portions of the fiber hyphae protruded. Along the pore wall surface scattered masses

of thin-walled, nodose-septate hyphae are found from which many basidia arose as terminal cells. The basidia and basidiospores that formed in the three types of cultures were similar in shape and size.

A comparison of the hyphal types and hyphal organization in the natural and cultural basidiocarps can be made. From the descriptions it is apparent that the cultural hyphae are similar in form to the hyphae of the natural basidiocarps. The skeletal hyphae are occasionally branched, thick-walled, aseptate, brownish-colored, and 1.7-3.9 μ in diameter. The fiber hyphae are aseptate, colorless or yellowish-brown, sparingly branched, and 1.7-4.4 μ in diameter. In their respective basidiocarps these hyphae are interwoven among each other in the pore walls and their hyphal tips protrude at the edge of the pore. The thin-walled generative hypha, and its counterpart, the thin-walled, nodose-septate hypha are found along the pore wall surface where they give rise to clavate, thin-walled basidia. The basidia give rise to narrow-cylindric, smooth-walled, 4.0-7.0 x 1.5-2.0 (-2.3) μ basidiospores. Irregularly thickened hyphae are infrequently observed in the pore walls of the natural basidiocarps and in the pore walls of older agar cultures. Nobles and Frew (1962) reported that in the fruiting areas of cultures of *Pycnoporus cinnabarinus* there were fiber hyphae, frequently in parallel arrangement, which were probably comparable to the skeletal hyphae of the context. In the *F. cajanderi* basidiocarps formed in

culture no parallel arrangement of fiber hyphae was noted.

C. Taxonomic considerations

Examination of over 90 collections of *F. cajanderi* has provided new information about the microstructure of the natural basidiocarps, but no new characters of the macrostructure were found. An evaluation of the descriptions of this species in the literature will be given in light of the information provided by this study. Since *F. cajanderi* and *F. roseus* are often confused because of their pink-colored pore surfaces and occasionally, their similar form, the latter species will also be examined in this discussion.

The descriptions of the macrostructure of basidiocarps of *F. cajanderi* in this study agree reasonably well with those in the literature. No new characters were found that extended the range of morphological features. The descriptions in the literature are of basidiocarps considered to be fully developed. It was noted in this study that young basidiocarps may be temporarily resupinate before assuming the effused-reflexed mature form. The resupinate form is not characteristic of the fully developed basidiocarps.

In many of the descriptions of *F. cajanderi* in the literature *F. roseus* is mentioned and various features are given to help distinguish the two species. An exception is the work of Murrill (1903), who thought that *F. cajanderi* and *F. roseus* were the same species. This

concept has been proven incorrect. Weir (1923) distinguished these two species by differences in context color, pileus surface features, and shape and size of spores. He also noted that *F. roseus* was found only on coniferous wood while *F. cajanderi* (as *Trametes subrosea*) was found on coniferous and angiospermous wood. Since that time there have been reports of basidiocarps of *F. roseus* growing on angiospermous wood. Bondartsev (1953) contends that *F. cajanderi* (as *Fomitopsis subrosea*) is easily distinguished from *F. roseus* by the thin, dimidiate pilei that never acquire the ungulate form, the zonate surface, and the radially flattened fibers of the former species. Variations of the *F. cajanderi* basidiocarps observed in this study do not allow these characters to be used with that degree of certainty. Bondartsev also states that the context is darker in *F. cajanderi* and the spores differ from those of *F. roseus*. According to Overholts (1953) *F. roseus* is readily distinguished from *F. cajanderi* by differences in spore shape and size. The basidiospores of *F. cajanderi* are narrower and slightly curved, measuring 4-7 x 1.5-2 μ, while in *F. roseus* they are elongate-ellipsoid to oblong, not at all curved, and measure 5-7 (-8) x 2.5-3.5 μ. These observations were confirmed by Mounce and Macrae (1937) who also applied the mating test and found these species to be completely intersterile. Similar measurements of *F. cajanderi* spores were obtained in this study, although infrequently the spores measured 2.3 μ wide. Another

distinguishing feature is the significant difference in growth rate at 30°C between the two species as reported by Snell, Hutchinson, and Newton (1928). To sum up, *F. cajanderi* can be positively distinguished from *F. roseus* by using any of three criteria: spore shape and size, growth rates, or mating tests. Field characters that can be used, but with less degree of confidence, are: the darker-colored context, the lack of any incrustation, and the often fused basidiocarps of *F. cajanderi*. Basidiocarps of *F. roseus* are solitary and never laterally fused in long bands like *F. cajanderi*.

The microstructure of *F. cajanderi* basidiocarps has not been examined in detail until this study. The hyphal descriptions in the literature are scanty and no information on hyphal development is given. A typical description of the hyphae found in *F. cajanderi* basidiocarps is that of Overholts (1953). He observed that in KOH solution the hyphae were pale brown, long and flexuous, simple, with no cross walls or clamps, 2.5-5 µ in diameter. Bondertsev (1953) recorded the hyphae as "faintly colored, rusty to light-brown in bulk, with very sparse branchings, thick-walled or even devoid of lumen, sharply outlined, (2-) 3-4.5 (-5.5) µ thick, in the tubes generally thinner and more sinuous..." In these descriptions the authors are describing skeletal hyphae. The sparse branchings noted by Bondartsev probably are those of the arboriform skeletal hyphae. A major hyphal type that is not mentioned in any

of the species descriptions of *F. cajanderi* is the presence of irregularly thickened generative hyphae. These hyphae are found abundantly throughout the context regions of the basidiocarps and are very characteristic because of their swollen clamp connections, short, solid protuberances along the hyphal walls, and their marked tendency to break at the clamp connections. The differentiation of these generative hyphae is characteristic of *F. cajanderi* and as such should be included in the species descriptions. Teixeira (1960) states, "The majority of descriptions of the hyphae of polypores refer to the skeletal and (or) binding hyphae. Most descriptions of dimitic or trimitic species completely ignore the generative hyphae." According to Teixeira, the importance of clamp connections on these hyphae is that it provides a clue to the phylogeny of the species. It is commonly accepted that clamp connections were present on members of the ancestral groups of Basidiomycetes. Thus species that still show clamp connections have retained the ancestral way of reproducing their dikaryotic cells.

The use of cultural characters in identification of polypore species has received much attention in recent years due to the work of Nobles. She has published keys by which wood-inhabiting polypores can be identified by a number of criteria. The diagnostic characters displayed by many fungi growing in culture are uniform and the microstructures produced are constant for each species (Nobles, 1971). The uniformity of hyphal types found in culture

also applies to the hyphae found in the natural basidiocarps. There is a striking homology between the hyphae produced in culture and in nature. It is well known that the complex basidiocarps of polypores are extremely difficult to examine in terms of the form and development of the hyphae present. By examining cultures of the same species the hyphal types may be more readily determined, since the mycelium produced in culture is much less difficult to dissect and observe. Comparative studies of cultures and natural basidiocarps would allow a better understanding of hyphal development in culture and in nature. Studies by van der Westhuizen (1963) and Nobles and Frew (1962) are two examples of where this has been done. More of this type of work is required in order to gain a broad range of morphological attributes upon which homogeneous, natural classifications of the Polyporaceae can be based.

It should be pointed out that Nobles (1971) believes the fiber hyphae produced in culture are homologous with the skeletal and binding hyphae of the natural basidiocarps and, in general, occur in those species where basidiocarp hyphal systems are described as dimitic or trimitic. The thin-walled, nodose-septate hyphae of Nobles' terminology are therefore homologous to the generative hyphae of Corner. It should also be noted that although the hyphae are homologous, one should not assume that the hyphae will be identical in every aspect of form and development. Slight variations in hyphal diameter, wall color and thickness,

number of branches, etc., can be expected. The hyphae in culture are growing under conditions of enriched nutrients, moist atmosphere, relatively constant temperature, and limited aeration. The hyphae in natural basidiocarps are subjected to the effects of weathering, desiccation, high light levels, fluctuating temperatures, and relatively high oxygen levels. These factors will influence the final morphology of the hyphae and thus minor differences may be noted between the hyphae produced in culture and in nature. However, this does not detract from the usefulness of cultural mycelium in providing basic information about hyphal development since, under similar cultural conditions, the hyphae show a remarkable homogeneity of form and development. This is exemplified by *F. cajanderi* in which the irregularly thickened generative hyphae of the natural basidiocarps are the same as the irregularly thickened, nodose-septate hyphae produced in culture.

In *F. cajanderi* the development of the mycelium in nature and in culture is essentially the same through the vegetative and reproductive phases. On the basis of this study one set of terms is all that is necessary for describing the development of both the natural and cultural mycelium because there is complete agreement between these phases as to hyphal structure and development. Therefore, I believe that the terms generative, irregularly thickened generative, and skeletal should be used to describe the kinds of hyphae. The descriptive phrases thin-walled,

nodose-septate, and fiber for hyphae developing in culture can be discarded. Thus, comparison of the structure and development of different polypore species will be made less difficult by this simplification of terminology.

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APPENDICES

APPENDIX I

List of *Fomes cajanderi* Collections

Collection No.	Substratum	Geographical location	Collection date
B-3	buried wood	Swan Hills, Alta.	Sept., 1970
B-12	conifer log	Crimson Lake, Alta.	June 20, 1971
B-19	spruce	Sundance Campsite near Edson, Alta.	July 5, 1971
B-20	spruce	Sundance Campsite near Edson, Alta.	July 5, 1971
B-22	spruce	Sundance Campsite near Edson, Alta.	July 5, 1971
B-23	conifer log	Sundance Campsite near Edson, Alta.	July 5, 1971
B-24	spruce	Forestry Trunk Road, 3 mi south of Highway 16, Alta.	July 5, 1971
B-25	pine	Forestry Trunk Road, 3 mi south of Highway 16, Alta.	July 5, 1971
B-27	spruce	Wolf Creek Campsite near Edson, Alta.	July 5, 1971
B-52	aspen poplar	Elk Island National Park, Alta.	Aug. 6, 1971
B-72	conifer log	Pembina Provincial Park, Entwhistle, Alta.	Aug. 16, 1971

Collection No.	Substratum	Geographical location	Collection date
B-74	spruce	Pembina Provincial Park, Entwhistle, Alta.	Aug. 16, 1971
B-92	spruce	Wolf Creek Campsite near Edson, Alta.	Aug. 16, 1971
B-110	conifer log	F.T.R., 9 mi south of Clearwater River, Alta.	Aug. 18, 1971
B-119	poplar	Campsite on Highway 11, 3 mi east of Alhambra turnoff, Alta.	Aug. 19, 1971
B-120	pine	James River Campsite near Sundre, Alta.	Aug. 19, 1971
B-126	spruce	Winterburn, Alta.	Aug. 24, 1971
B-127	spruce	Winterburn, Alta.	Aug. 24, 1971
B-128	spruce	Spruce bog 3 mi west of Carvel Corner, Highway 16, Alta.	Aug. 24, 1971
B-128a	spruce	Spruce bog 3 mi west of Carvel Corner, Highway 16, Alta.	Aug. 24, 1971
B-139	spruce	Blackmud Creek, Edmonton, Alta.	Aug. 27, 1971
B-166	spruce	Wabasca road, 32 mi north of Slave Lake, Alta.	Aug. 31, 1971

Collection No.	Substratum	Geographical location	Collection date
B-170	spruce	Wabasca road, 32 mi north of Slave Lake, Alta.	Aug. 31, 1971
B-186	spruce	40 mi south of Fort Nelson B.C.	Aug. 29, 1971
B-187	spruce	Martin Creek, 140 mi north of Wonowon, B.C.	Aug. 29, 1971
B-188	spruce	Kledo River Campsite, Alaska Highway, B.C.	Aug. 29, 1971
B-189	pine	30 mi north of Prophet River, B.C.	Aug. 28, 1971
B-191	pine	12 mi northwest of Dawson Creek, B.C.	Aug. 27, 1971
B-192	spruce	20 mi north of Wonowon, B.C.	Aug. 27, 1971
B-193	conifer log	5 mi north of Wonowon, B.C.	Aug. 27, 1971
B-194	spruce	5 mi north of Wonowon, B.C.	Aug. 27, 1971
B-195	conifer log	5 mi north of Wonowon, B.C.	Aug. 27, 1971
B-196	spruce	5 mi north of Wonowon, B.C.	Aug. 27, 1971
B-198	pine	12 mi northwest of Dawson Creek, B.C.	Aug. 27, 1971
B-201	spruce	7 mi east of Prophet River, B.C.	Aug. 28, 1971

Collection No.	Substratum	Geographical location	Collection date
B-202	spruce	7 mi south of Prophet River, B.C.	Aug. 28, 1971
B-286	spruce	Carson Lake, north of Whitecourt, Alta.	July 6, 1972
B-288	spruce	Carson Lake, north of Whitecourt, Alta.	July 6, 1972
B-289	spruce	Carson Lake, north of Whitecourt, Alta.	July 6, 1972
B-291	spruce	Carson Lake, north of Whitecourt, Alta.	July 6, 1972
B-294	spruce	Carson Lake, north of Whitecourt, Alta.	July 6, 1972
B-295	spruce	Smoky Tower, 67 mi south of Grande Prairie, Alta.	July 8, 1972
B-296	conifer log	Spruce bog 3 mi west of Carvel Corner, Highway 16, Alta.	July 11, 1972
B-321	spruce	Carson Lake, north of Whitecourt, Alta.	Aug. 4, 1972
B-322	spruce	Carson Lake, north of Whitecourt, Alta.	Aug. 4, 1972
B-324	spruce	Carson Lake, north of Whitecourt, Alta.	Aug. 4, 1972

Collection No.	Substratum	Geographical location	Collection date
B-325	spruce	Carson Lake, north of Whitecourt, Alta.	Aug. 4, 1972
B-326	spruce	Carson Lake, north of Whitecourt, Alta.	Aug. 4, 1972
B-333	spruce	MacKenzie Ravine, Edmonton, Alta.	Oct. 9, 1972

APPENDIX II

Staining Schedule for Decayed Wood Sections

(modified from Cartwright, 1929)

1.	1% aqueous safranin	30-45 sec
2.	Wash in distilled water	
3.	Heat gently over alcohol lamp with picro-aniline blue	2-3 min
4.	Wash in distilled water	
5.	Ethanol series - 50%	1/2-1 min
	70%	"
	85%	"
	98%	"
6.	Clear in clove oil	2-3 min
7.	Wash twice in xylene	
8.	Mount in Kleermount	

The wood cells stain red while the mycelium stains blue.

APPENDIX III

Basal Synthetic Medium

(Jennison, Newcomb, and Henderson, 1955)

Glucose	100 gm
Glutamic acid	800 mg total nitrogen
KH_2PO_4	6.0 gm
$MgSO_4 \cdot 7H_2O$	0.4 gm
Thiamine hydrochloride	2.5 mg

Trace elements: B, 0.10 mg; Cu, 0.01 mg; Fe, 0.05 mg; Mn, 0.01 mg; Mo, 0.01 mg; Zn, 0.07 mg.

Distilled water to make 1 liter.

Initial pH 4.2

Modification: (1) 10 gm amounts of carbon sources substituted for glucose.

(2) equivalent amount of nitrogen source as L-asparagine or DL-asparagine substituted for glutamic acid.

(3) initial pH after sterilization 4.7-5.0.

Nobles' Malt Agar

(Nobles, 1948)

Difco Bacto malt extract	12.5 gm
Difco Bacto-agar	20.0 gm
Distilled water	1000.0 ml

B30070